REMARKS

Claims 1-30 are currently pending.

I. Request for Interview

In June, 2008, Applicant's undersigned representative left voice mail messages with Examiner Clark to request an interview. However, the Examiner did not return the voice mail messages. Applicant is still interested in conducting an interview to discuss the amendments to the claims and Applicant's data.

II. The Objection to the Title

The Examiner states that the title of the invention is not descriptive and suggests the title be amended to "A composition obtained from barley shochu stillage".

The title has been amended as suggested by the Examiner. It is respectfully submitted that Applicant's abstract fully complies with 37 C.F.R. §1.72(a) and MPEP §606 and it is requested that the objection to the title be reconsidered and withdrawn.

III. The Objections to Claims 1, 28 and 29

Claims 1, 28 and 29 are objected to as allegedly containing "informalities".

The Examiner requests that Applicant amend claims 1, 28 and 29 to delete the term "of" from between the percent and the amino acid or sugar.

Claims 1, 28 and 29 have been amended as suggested by the Examiner. It is respectfully submitted that Applicant's claims fully complies with 35 U.S.C. § 112 and it is requested that the objection to the claims be reconsidered and withdrawn.

IV. The Rejection under 35 U.S.C. 112, First Paragraph

Claim 27 is rejected under 35 U.S.C. 112, first paragraph, because the specification allegedly does not reasonably provide enablement for a composition capable of treating the onset of alcoholic hepatopathy in a patient in need thereof.

The Examiner's position remains similar to that as previously set forth.

Further to the Examiner's position concerning the conclusions to be found based on testing on rats, Applicant previously submitted three journal articles. There are numerous patent publications provide support the fact that there is a correlation between (rat) experiment and effect on human beings (that experimentally, there is a high correlation between data from animal experiments and test result on human beings, and that it can sufficiently be applied). Applicant has researched some examples of where patent was obtained with animal testing alone. Listed below are a sample of patent documents that relate to the suppression of hepatopathy, and which only lists animal experimentation as Examples. See the attachments to this Amendment. Japanese Patent No. 3209375 (Abstract and Machine language translation), Japanese Patent No. 2821575 (Abstract), Japanese Patent No. 4104853 (submitted as U.S. Patent Application Publication US2002/110605), Japanese Patent No. 2919870 (submitted as U.S. Patent 5081149), Japanese Patent No. 3207523 (Abstract and Machine language translation) and Japanese Patent No. 3090980 (Abstract and Machine language translation). Applicant respectfully submits that it is well established that the testing on rats can used as a correlation for effect on human beings.

To advance prosecution, Applicant has amended claim 27 to change "treating the onset of alcoholic hepatopathy in a patient in need thereof" to "treating the onset of alcoholic hepatopathy

in a patient in need thereof. Applicant has also added new claim 30 to recite "preventing the on-set of alcoholic fatty liver in a patient in need thereof." Support for the amendment to claim 27 and new claim 30 may be found in the specification as originally filed, for example, page 2, first full paragraph.

Applicant's respectfully submit that Applicant's specification is fully enabling for a composition that is capable of preventing the on-set and treatment of reversible fatty liver at a comparatively early-stage of hepatopathy caused by alcohol. As for the treatment of alcoholic fatty liver, please see Test Example 2 and 3 of Applicant's specification.

Regarding experimental data that indicates preventive effect, a relevant experiment may be found in Test Example 1 of Applicant's specification. Test Example 1 administers each experiment sample together with 5% alcohol to rats that have been administered alcohol for 6 days. For non-treated control group, alcohol is not administered, and for control group, only 5% alcohol is administered, and for each experiment group A through D, each experiment sample is administered with 5% alcohol.

As a result, when composition matter (Group A) indicated in this invention is administered, increase in serum LDL-cholesterol concentration, serum triglyceride concentration, and liver triglyceride concentration are inhibited significantly, and a tendency of slightly decreasing the necrosis of hepatocytes and the balloon-like swelling in the terminal hepatic vein peripheral region of the hepatic lobule in the observation of the hepatocytes through the biological microscope. From this, it is concluded that the onset of the alcoholic fatty liver is significantly inhibited, indicating a strong suppression of onset against the alcoholic fatty liver.

The above data is submitted in Applicant's specification as filed and therefore it is not necessary to resubmit the data in a 132 Declaration.

Applicant submits that their disclosure is fully enabling and request that the rejection under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn. It is respectfully submitted that one of ordinary skill in the art would be able to practice Applicant's invention without undue experimentation.

V. The Rejection of Claim 2 under 35 U.S.C. 112, Second Paragraph

The Examiner's position is that there is no mention of "free amino acids" and "amino acids in said peptides" prior to the use of the terms "the free amino acids" and "the amino acids in said peptides"

Claim 2 has been amended to recite: "the basis of total weight of free amino acids and amino acids in said peptides."

It is respectfully submitted that Applicant's claims are clear and definite and it is requested that the rejection under 35 U.S.C. §112 be reconsidered and withdrawn.

VI. The Rejection Based on Omori et al.

Claims 1-7, 28 and 29 are rejected under 35 U.S.C. 102(b) as allegedly being anticipated by "Omori et al. (N, JP 2000-125777 A, Translation provided herein)".

First of all, the Examiner confirmed in a telephone interview that the Examiner meant to reject the claims based on Omori et al JP2000/342247. This is reference listed as "N" in the on the Examiner's Form PTO SB/08. JP2000/125777 is to unrelated subject matter.

The Examiner alleges that Omori teaches a method of obtaining a composition that is "one and the same as disclosed in the instantly claimed invention of Applicant." Thus, the

Examiner alleges that a composition obtained by solid/liquid separation of shochu stillage taught by Omori, which is alleged to recite the same method steps as claimed by Applicant to provide a product-by-process, inherently contains the components in the ranges claimed by Applicant.

Applicant respectfully submits that the present invention is not anticipated by or obvious over the disclosures of Omori and request that the Examiner reconsider and withdraw this rejection in view of the following remarks.

Applicant's specifically note that there is a difference between the production method of this application and Omori (JP2000/342247) hereinafter the "reference".

As explained in further detail below, the difference is that in the reference, it is indicated that before the synthetic adsorbent process, a condensation step is perfomred (Reference example [0009], third step, [0012] the third step, [Example 1 through 3] etc.). In the manufacturing process of the present application, there is no indication relating to such a condensation.

Therefore, the manufacturing processes differ on this point.

If synthetic absorbent process is conducted here, the composition of the composition matter that absorbs and the composition of the composition matter that pass through would differ because of the difference in the concentration of the liquid passing through. Therefore, the composition matter obtained, as written in the reference, by the synthetic absorbent process conducted on a state where the condensation process has been conducted to reach approximately three (3) times the concentration is different from the composition matter of the present Application, where the synthetic absorbent process is conducted with no condensation.

Example 1 of the present application is an example of the inventive composition:

The barley shochu stillage obtained was centrifuged under conditions of 8,000 rpm and 10 minutes to form a liquid fraction of the barley shochu stillage, and 25 L of the liquid fraction and 10 L of deionized water were passed in this order through a column (resin volume 10 L) filled with a synthetic adsorbent Amberlite XAD-16 manufactured by Organo K.K. to obtain an unadsorbed fraction comprising a bypassed solution showing an unadsorbability to the synthetic adsorbent of the column. The resulting unadsorbed fraction was freeze-dried with a vacuum freeze-dryer to obtain 1,200 g of a freeze-dried product. The resulting freeze-dried product was pulverized to give a light yellow powder.

From the method of producing a composition indicated in the reference (emphasis added):

(Example 1 of the reference):

A part for the fluid obtained by carrying out solid liquid separation of said 1 kl of shochu distillation residual liquor made from barley obtained by the distillation process of the white-distilled-liquor manufacture made from barley with the solid-liquid-separation machine of the screw press method made from Nobukazu Engineering the filtration squeezer further by Yabuta Industrial Company. It used, a part for SS was separated further, and about 0.85 kl of clear liquid was obtained. Next, this clear liquid was condensed to about 1/3 concentrate using the Ogawara Factory vacuum evaporator. This obtained concentrate was contacted in the column filled up with Mitsubishi Chemical 850 [synthetic adsorbent material SEPABIZU SP], and the non-adsorptivity fraction which shows non-adsorptivity to the synthetic adsorbent material concerned eluted from the column concerned was obtained. The product spray type dryer made from

Ogawara Chemical engineering machine is used for the obtained non-adsorptivity fraction, Inlet temperature 150 °C, the outlet temperature of 80°C, amount of 0.45 m^3/min of hot winds, and min, and the volume of atomizing air Spray drying was carried out on condition of 1.0 kgf/cm^2, and about 10-kg powdered dry matter for culture media used for the culture medium for microorganisms was obtained.

(Example 2 of the reference):

A part for the fluid obtained by separating solid-liquid with the solid-liquid-separation machine of the screw press method made from Nobukazu Engineering in said 1 kl of shochu distillation residual liquor made from barley obtained by the distillation process of the white-distilled-liquor manufacture made from barley further the Tomoe Engineering decanter type centrifuge. A part for SS was further separated for a part for the fluid obtained by using and separating solid-liquid using the ceramic filtration apparatus further by Japanese Schumacher, about 0.85 kl of clear liquid was obtained, the clear liquid concerned was neutralized by sodium hydroxide, and about 0.9 kl of neutralization liquid was obtained. Next, condense the neutralization liquid concerned up to about 1/3 concentrate using the Ogawara Factory vacuum evaporator. The obtained concentrate concerned is contacted in the column filled up with Mitsubishi Chemical 850 [synthetic adsorbent material SEPABIZU SP]. The non-adsorptivity fraction which shows non-adsorptivity to the synthetic adsorbent material concerned eluted from the packed column concerned is obtained. The product spray type dryer made from Ogawara Chemical engineering machine is used for the obtained non-adsorptivity fraction, Inlet temperature 150 °C, the outlet temperature of 80 °C, amount of 0.45 m^3/min of hot winds, and

min, and the volume of atomizing air Spray drying was carried out on condition of 1.0 kgf/cm^2, and about 8-kg dry matter for culture media used for the culture medium for microorganisms was obtained.

[Example 3 of the reference]

A part for the fluid obtained by separating solid-liquid with the solid-liquid-separation machine of the screw press method made from Nobukazu Engineering in said 1 kl of shochu distillation residual liquor made from barley obtained by the distillation process of the white-distilled-liquor manufacture made from barley further the Tomoe Engineering decanter type centrifuge. It used, solid-liquid was separated and a part for about 0.85-kl fluid was obtained. Next, condense to about 1/3 concentrate for the fluid concerned using the Ogawara Factory vacuum evaporator, and a concentrate is obtained. The obtained concentrate concerned is contacted in the column filled up with Mitsubishi Chemical 850 [synthetic adsorbent material SEPABIZU SP]. The non-adsorptivity fraction which shows non-adsorptivity to the synthetic adsorbent material concerned eluted from the packed column concerned is obtained. They are extractives to the obtained non-adsorptivity fraction. 1.5-times the amount dextrin friend call for the product foodstuffs addition made from Japanese Dregs Chemicals The product spray type dryer made from Ogawara Chemical engineering machine is used for 6-L after addition, Inlet temperature 150 °C, the outlet temperature of 80 °C, amount of 0.45 m^3/min of hot winds, and min, and the volume of atomizing air Spray drying was carried out on condition of 1.0 kgf/cm^2, and about 12-kg dry matter used for the culture medium for microorganisms was obtained.

As discussed above, it is clear that the compositions themselves are different due to the different production methods. In the present application, the synthetic absorbent process is conducted on a state of concentrate solution as is with no condensation, while the reference conducts the synthetic absorbent process is conducted after condensed to about 3 times.

Further, there is a difference between the two in that the synthetic absorbent used in Example are different in type, where this application uses Amberlite XAD-16 manufactured by Organo K.K., the reference uses Mitsubishi Chemical 850 [synthetic adsorbent material SEPABIZU SP].

The above Examples are from the reference and therefore it is not believed that a 132 Declaration is necessary. However, Applicant will resubmit the production methods of the reference in the form of a 132 declaration if deemed necessary by the Examiner.

Noting the above differences in the production methods of Applicant's specification versus those of the reference, Applicant respectfully disagrees with the Examiner's allegation that the claimed composition of the reference are "one and the same as disclosed in the instantly claimed invention of Applicant" and Applicant respectfully disagrees that the reference recites the same method steps and thus inherently contains the components in the ranges claimed by Applicant.

For the above reasons, it is respectfully submitted that the subject matter of claims 1-7, 28 and 29 is neither taught by nor made obvious from the disclosures of Omori et al and it is requested that the rejection under 35 U.S.C. §102 be reconsidered and withdrawn.

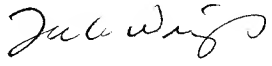
VII. Conclusion

In view of the above, Applicant respectfully submits that their claimed invention is allowable and ask that the objection to the specification and the claims, the rejections under 35 U.S.C. §112, and the rejection under 35 U.S.C. §102 be reconsidered and withdrawn. Applicant respectfully submits that this case is in condition for allowance and allowance is respectfully solicited.

If this paper is not timely filed, Applicant respectfully petitions for an appropriate extension of time. The fees for such an extension or any other fees that may be due with respect to this paper may be charged to Deposit Account No. 50-2866.

Respectfully submitted,

WESTERMAN, HATTORI, DANIELS & ADRIAN, LLP



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Enclosures:

Japanese Patent No. 3209375 (Abstract and Machine language translation)
Japanese Patent No. 2821575 (Abstract),
U.S. Patent Application Publication US2002/110605,
U.S. Patent 5081149,
Japanese Patent No. 3207523 (Abstract and Machine language translation) and
Japanese Patent No. 3090980 (Abstract and Machine language translation).

ADDITIVE OF STYRENES OLIGOMER, EPOXY RESIN CURING AGENT COMPRISING SAME ADDITIVE AS CONSTITUENT COMPONENT AND RESIN COMPOSITION

Publication number: JP3209375
Publication date: 1991-09-12
Inventor: NARUSE YOSHIHIRO; YAMAMOTO SEIJI; KAJIOKA MASAHIKO
Applicant: KAWASAKI STEEL CO
Classification:
- international: ***C07D307/60; C08F299/04; C08G59/42; C08L67/06;*** **C07D307/00; C08F299/00; C08G59/00; C08L67/00;** (IPC1-7): C07D307/60; C08F299/04; C08G59/42; C08L67/06
- European:
Application number: JP19890289649 19891107
Priority number(s): JP19890289649 19891107; JP19880279464 19881107

Abstract of **JP3209375**

PURPOSE:To obtain an adduct of styrene oligomer useful as a curing agent for epoxy resins by adding an unsaturated dicarboxylic acid anhydride to styrene oligomer. CONSTITUTION:An unsaturated dicarboxylic acid anhydride (e.g. maleic anhydride) is added to a low-molecular weight styrene oligomer, preferably having >=20g-I2/100g iodine value and <=1,000 average molecular weight in an inert gas atmosphere by blending under heating at 180-240 deg.C to give the objective substance. >=50mol% unsaturated dicarboxylic acid anhydride is added to 1mol unsaturated double bond of the styrene oligomer. The adduct can be made from a pasty state to a solid state by changing an amount of the unsaturated dicarboxylic acid anhydride to be added and the additive shows excellent compatibility with a highly polar resin.

CLAIMS

[Claim(s)]
[Claim 1]An alcoholic-liver-injury mitigating agent which makes gelatin or soluble collagen an active principle.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the alcoholic-liver-injury mitigating agent which makes gelatin or soluble collagen an active principle.
[0002]
[Description of the Prior Art]It is known that the function of liver has much operations, such as the metabolism of a detoxifying effect, sugar, protein, or a fat, cholepoiesis and secretion, a hormonal adjustment operation, a generation operation of a blood coagulation substance (prothrombin), a storage operation of various living body components, and a regenerative action of hepatocytes. However, the function of liver may receive an obstacle by the factor of alcohol, malnutrition, virus, drug, poison, and others versatility. And although there are dramatically many patients who received such an obstacle when a potential person is also included, a completely effective cure is in the state which has not yet been established. It is reported that mitigation or the curative effect of affection of the liver has various substances in order to improve this obstacle. For example, a KAMIGURE knurl (JP,60-258115,A), alpha-hydroxycarboxylic acid which has a chroman skeleton like 2-hydroxy-3-(3,4-dihydro-6-hydroxy-2,5,7,8- tetramethyl 2H-benzo bilane -2-yl) propionic acid (JP,61-204122,A), Guanidinoethane sulfonic acid (JP,62-138426,A), 2-(1,3- JICHIETAN -2-ylidyne)-5,5-dimethyl Substitution like a-1,3-cyclohexanedione -1,3-JICHIETAN derivative (JP,62-158214,A), Saponins (JP,3-31296,A), 2,2' - There are compounds, such as dithioscrew benzimidazole (JP,4-208223,A) and a methylenedioxy aniline derivative (JP,4-193828,A), etc. However, these compounds are chemicals although there is a plant component in a part.
It can necessarily be satisfied with neither safety when chronic dosing is carried out, nor the field of an effect. On the other hand, alcoholic ingestion is routinizing, as a result, various obstacles by alcoholic ingestion, especially alcoholic liver injury have been a problem, and the measure is desired in recent years.
[0003]
[Problem(s) to be Solved by the Invention]This invention persons were used for prevention mitigation of the condition of various obstacles by alcoholic ingestion, especially alcoholic liver injury, or recovery, and their safety was high and they performed search of the active principle which can be supplied cheaply and in large quantities over many years. For this reason, when various ingredients which exist naturally were examined, it finds out that gelatin or soluble collagen acts to reduce alcoholic liver injury, and came to complete this invention. That is, the technical problem of this invention has high safety, and it is in providing the alcoholic hepatopathy mitigating agent which can be obtained cheaply and in large quantities.
[0004]
[Means for Solving the Problem]As this invention persons are described above, in order that an ingredient effective in a therapy of alcoholic liver injury may be got, When an animal examination was done, relation between protein and alcoholic liver injury was considered and gelatin or soluble collagen, and alcohol were made to take in simultaneously, it finds out that there is an operation which makes alcoholic liver injury reduce, and came to complete this invention. That is, this invention relates to an alcoholic-liver-injury mitigating agent which makes gelatin or soluble collagen an active principle.
[0005]Gelatin of an active principle of this invention is a proteinic kind which refined glue taken from a hide and a bone of an animal, and is used for many foodstuffs. Collagen which is one of the vital-structures protein in a living body solubilizes protein which constitutes gelatin, and some collagen molecules understand it. What was solubilized without disassembling a collagen molecule is called soluble collagen. Gelatin and soluble collagen have the feature that many glycines, arginine, prolines, and hydroxyproline which are kinds of amino acid are included. An operation to which a glycine controls a rise of a blood cholesterol level of a rat which made more methionine than the amino acid amount required take in is known. [Essential amino acid research, 102, 20-25 (1984)] .Arginine taken in changes to an alanine in the living body, and it is known that this alanine is typical glyconeogenesis nature amino acid. [The Harper work, "biochemistry" (21st edition) 298 (1988)], .However, a thing [as / in this invention] for which gelatin and soluble collagen have an alcoholic-liver-injury mitigation operation is not found out. What is usually marketed can be used for such gelatin and

soluble collagen. "The gelatin MJ" (made by Nitta Gelatin, Inc., Inc.), the "gelatin S-1" (made by Nippy company), etc. are one of commercial gelatin, and there is "collagen powder PK" (made by Nitta Gelatin, Inc., Inc.) etc. in soluble collagen.
[0006]These gelatin and soluble collagen are made into a pharmaceutical form remaining as it is as powder, or suitable, can be taken in in taking orally, or can be heated and solated, or can be cooled further, can gel it, and can take in it in taking orally. Depending on Homo sapiens or the case, an animal is medicated as a dosage form suitable for other taking orally.
[0007]As an orally administered drug, there are a tablet, a capsule, a granule, subtle granules, powder, etc., Binding materials, such as the usual medicine manufacture adjuvant, for example, sorbitol, and a polyvinyl pyrrolidone, It pharmaceutical-preparation-izes with the usual pharmaceutical preparation technique with wetting agents, such as disintegrator, such as lubricant, such as excipients, such as lactose and cornstarch, magnesium stearate, and talc, potato starch, and carboxymethyl cellulose, and lauryl acid sodium, etc. A tablet can also be coated if needed.
[0008]Since an active principle of this invention is gelatin or soluble collagen, there is almost no acute toxicity, therefore this as it is, Or various nutritional information, a seasoning ingredient, a flavor component, a coloring component, etc. can be added, and it can mold into a suitable form, and can also use as functional food, a food for specified health use, and health food as an eating-and-drinking article. There are various vitamins and minerals in nutritional information, there are sucrose, L-glutamic acid, inosinic acid, citrate, etc. in a seasoning ingredient, and a coloring agent of foodstuffs is used for a coloring component for various kinds of flavors again at a flavor component.
[0009]Since these ingredients of safety are very high and a dose of gelatin of an active principle or soluble collagen can be supplied cheaply and in large quantities, if it is more than an effective dose, there will be no maximum in particular, but 50-500 mg per adult-man one-day weight kg is usually suitable. There is no hepatopathy mitigation operation that it is 50 mg or less, and even if it raises the dose as it is not less than 500 mg, a mitigation operation does not increase. In order to show a hepatopathy mitigation operation of this invention, the next animal examination was done.
[0010]Gelatin used the Wako Pure Chem make. After carrying out preliminary breeding of the 4-weeks old Wistar system male rat (CLEA Japan, Inc.) for five days with reference solution object feed which does not contain alcohol, one groups [eight] divided into each two groups, experiment feed as shown in Table 1 was paid, and it bred for five weeks. A ratio of gelatin of examination feed to casein was set to 3 to 7. In order to keep an essential amino acid of examination feed from running short, it supplemented, respectively so that each essential amino acid content in feed might amount to two thirds of Miyazaki patterns. A gamma-GTP value in a blood serum makes an incision in the abdomen a rat under pentobarbital sodium anesthesia after an end of breeding, It collected blood from back vena cava, a blood serum was separated, and it measured with enzymatic process (it measures using "DETAMINA gamma-GTP" Hiroshi Maruyama et al., the newest inspection, 5 and 87 (1987), and by Kyowa Medex [Co., Ltd.] Co., Ltd.). Component composition of feed used for an animal experiment is shown in Table 1, and a gamma-GTP value in a blood serum after experiment feed administration is shown in drawing 1. As shown in this figure, a gamma-GTP value of an examination group which prescribed gelatin for the patient was falling intentionally compared with a control group which is not prescribed for the patient, and it became clear from this that gelatin was effective in mitigation of condition of alcoholic liver injury. Although the same method as the above was enforced using soluble collagen ("collagen powder PK" by Nitta Gelatin, Inc., Inc.), almost same result was obtained and soluble collagen as well as gelatin was effective in alcoholic-liver-injury mitigation.
[0011]
[Table 1]
Examination feed presentation (g/L)
----------. contrast feed Examination feed . ----------. Corn oil 38.0000 38.0000. Corn starch 33.9000 33.9000. Vitamin mixing 0.0853 0.0853. salts mixing 7.7177 7.7177 -- 2 tartaric-acid Kolin . 0.5300 0.5300 Ethyl alcohol (99.5%) 50.7714 50.7714 Casein 47.6000 31.7300 Gelatin 0.0000 17.7500 Methionine 0.0000 0.2590. Cystein 0.7640 0.9060 Phenylalanine 0.0000 0.2250 Tryptophan 0.0000 0.1220 Monoglyceride 4.0000 4.0000. Methionine of an essential amino acid which is ---------- and which gelatin runs short of since examination feed added gelatin, phenylalanine, and tryptophan were added. Since cystein of casein origin decreased, cystein was increased so that a cystein content might become equal to contrast feed.
[0012]Next, an example is shown and this invention is explained concretely.
[0013]
[Example 1] It is lactose to 100 g of gelatin powder (made by Wako Pure Chem). 250 g may be added, and it mixes and is potato starch further. 150 g, magnesium stearate 3 g was added, it tableted with the tableting machine, and the tablet was obtained.
[0014]

[Example 2] To 100 g of soluble collagen powder ("collagen powder PK" by Nitta Gelatin, Inc.), it is sucrose. 150 g, lactose 250 g was added and squeezed and the stick shape solid was obtained.

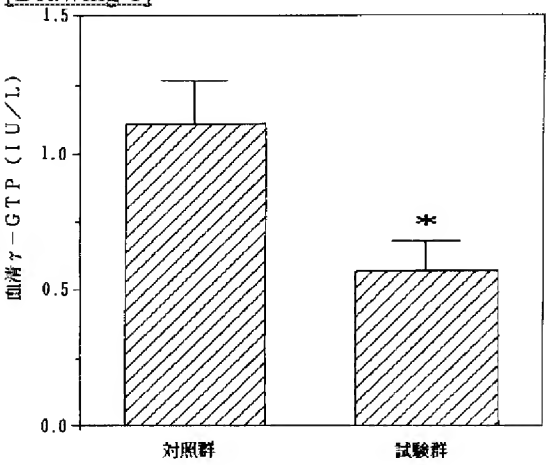
[0015]

[Effect of the Invention]Alcoholic liver injury is effectively mitigable by prescribing the protein originating in the hides and bones of an animal, such as gelatin and soluble collagen, for the patient in taking orally. In particular, since active principles are gelatin and soluble collagen, safety is very high and a medicine can be prescribed for the patient cheaply and in large quantities for a long period of time.

* NOTICES *

DRAWINGS

[Drawing 1]



[Translation done.]

LIVER DISORDER IMPROVER

Publication number: JP2069416
Publication date: 1990-03-08
Inventor: TANAKA MORIHISA; OTSUKA TAKAO; CHIYOU SOUTETSU
Applicant: TSUMURA & CO; KITASATO INST
Classification:
- international: ***C07D491/056; A61K31/435; A61K31/535; A61K36/18; A61K36/28; A61K36/73; A61P1/16; C07D455/03;*** **C07D491/00; A61K31/435; A61K31/535; A61K36/18; A61K36/185; A61P1/00; C07D455/00;** (IPC1-7): A61K31/435; A61K31/535; A61K35/78; C07D455/03; C07D491/056
- European:
Application number: JP19880220347 19880905
Priority number(s): JP19880220347 19880905

Abstract of **JP2069416**

PURPOSE:To obtain a liver disorder improver comprising an extract of Inulae flos, an extract of Cyananchi atrati radix or berberine as an active ingredient. CONSTITUTION:Inulae flos or Cyananchi atrati radix, Japanese Chinese crude drug is extracted with water and/or an organic solvent while warming and the solvent is distilled away under reduced pressure to give an extract of Inulae flos or Cyananchi atrati radix. The extract or berberine is properly blended with a conventional excipient, a binder, a disintegrator, a surfactant, a lubricant, a fluidity promoter, etc., and pharmaceutically manufactured by a conventional procedure to give the aimed substance. The substance can be prepared into a dosage form such as tablet, capsule, granule, fine granule, powder, injection, suppository, etc. A dose is 1-50g calculated as weight of extract of Inulae flos or Cyananchi atrati radix or 10-300g berberine per adult daily in the case of oral agent and administered dividedly several times.

US 20020110605A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2002/0110605 A1**

Nakagiri et al. (43) **Pub. Date: Aug. 15, 2002**

(54) **LIVER FUNCTION PROTECTING OR IMPROVING AGENT**

(76) Inventors: **Ryusuke Nakagiri**, Tsukuba-shi (JP); **Toshikazu Kamiya**, Tsukuba-shi (JP); **Erika Hashizume**, Tsukuba-shi (JP); **Yasushi Sakai**, Inashiki-gun (JP); **Shun Kayahashi**, Tsukuba-shi (JP)

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(21) Appl. No.: **10/010,154**

(22) Filed: **Dec. 10, 2001**

(30) **Foreign Application Priority Data**

Dec. 11, 2000 (JP) 375510

Publication Classification

(51) **Int. Cl.**[7] **A61K 35/78**

(52) **U.S. Cl.** **424/725**

(57) **ABSTRACT**

The present invention provides a liver function protecting or improving agent, foods and drinks or feeds having liver function protecting or improving activity, and additives for foods and drinks or feeds having liver function protecting or improving activity, which comprise a plant of the family Saxifragaceae or an extract of the plant. Also provided is a method of screening for liver function protecting or improving agents.

LIVER FUNCTION PROTECTING OR IMPROVING AGENT

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a liver function protecting or improving agent, foods and drinks or feeds having liver function protecting or improving activity, additives for foods and drinks or for feeds having liver function protecting or improving activity, a method of screening for liver function protecting or improving agents, and a method of protecting or improving the animal liver function.

[0002] The liver is an important organ which has various functions such as metabolic regulation and storage of sugar, protein and lipid which are three major nutrients, and decomposition and detoxification of substances unnecessary to the body. These functions suffer acute or chronic disorders due to an excessive intake of alcohol, viral infection, bad eating habits, stress, smoking, etc. The advance of these disorders brings about diseases such as acute hepatitis, chronic hepatitis, hepatic cirrhosis, alcoholic fatty liver, hepatitis B and liver cancer.

[0003] When liver cells are damaged by virus, alcohol, etc., enzymes such as aspartate aminotransferase (glutamic-oxaloacetic transaminase, hereinafter abbreviated as GOT) and alanine aminotransferase (glutamic-pyruvic transaminase, hereinafter abbreviated as GPT) in the cells leak into the blood, which raises the values indicating the activities of these enzymes. Accordingly, the levels of GOT and GPT activities in the blood are known to indicate the levels of the liver function disorders.

[0004] Known drugs used for the protection or treatment of the liver function disorders include antiviral agents such as acyclovir, immunosuppressive agents (Journal of Clinical and Experimental Medicine, Vol. 171, No. 14, 957-1158, Ishiyaku Pub., Inc., 1994), and glutathione (Protein, Nucleic Acid and Enzyme, Vol. 33, No. 9, 1625-1631, Kyoritsu Shuppan Co., Ltd., 1988). Foods and drinks which are recognized to be effective for protecting, strengthening and improving the liver function include turmeric, milk thistle, sesame lignan, oyster extract and liver extract (Food Style 21, Vol. 2, No. 12, Shokuhin Kagaku Shinbunsha, 1998).

[0005] *Hydrangea macrophylla* Seringe var. Thunbergii Makino, which is a plant of the family Saxifragaceae, is a species akin to hydrangea and said to have been developed in the process of breeding *Hydrangea macrophylla* Seringe var. acuminata. Hydrangeae Dulcis Folium, which is prepared by fermenting leaves and branch ends of *Hydrangea macrophylla* Seringe var. Thunbergii Makino, followed by drying, is known as a crude drug produced in Japan.

[0006] Hydrangeae Dulcis Folium and an extract of Hydrangeae Dulcis Folium have been conventionally used as starting materials for correctives (sweeteners) in pills and for mouth refreshers (The Japanese Pharmacopoeia, 12th revision, D-31-33, Hirokawa Pub., Co., Ltd., 1991). The extract of Hydrangeae Dulcis Folium, which is a food additive (sweetener), is obtained by extracting leaves of Hydrangeae Dulcis Folium with water. It is used in the form of a liquid or a powder obtained by concentration, and its sweetening component is phyllodulcin (Existing Additives and Natural Flavors, Food Materials "Natural Products Handbook" 14th edition, Shokuhin to Kagaku, 1998).

[0007] Hydrangeae Dulcis Folium is known to have anti-Coccidium activity, anti-fungal activity, anti-ulcer activity, anti-allergic activity, hypercholesterolemia suppressing activity, anti-periodontal bacteria activity, anti-oxidation activity, and the like (Summary of Lectures at the 2nd Symposium on Medicines and Foods, p. 85, 1999). The extract of Hydrangeae Dulcis Folium is known to have cholagoic activity (Journal of the Pharmaceutical Society of Japan, Vol. 114, No. 6, 401-413, 1994). The extract of Hydrangeae Dulcis Folium is also known to exhibit suppressing activity on in vitro lipid peroxidation reaction of liver microsomes [Natural Medicines, 49(1), 84-87, 1995].

[0008] However, there has been no report that plants of the family Saxifragaceae such as Hydrangeae Dulcis Folium and *Saxifraga stolonifera* Meerb. have liver function protecting or improving activity.

[0009] Screening methods for liver function protecting or improving agents are known which use rodents having the blood GPT and GOT levels raised by administration of D-galactosamine [Journal of Nutrition, 129, 1361 (1999)], acetaminophen [Planta Medica, 55, 417 (1989)] and carbon tetrachloride [Fundamental & Clinical Pharmacology, 3, 183 (1989)].

[0010] On the other hand, it is known that GPT and GOT activities in the blood can be enhanced by administration of ethanol, followed by administration of lipopolysaccharide [Gastroenterology, 115, 443 (1998)]. By this method, hepatopathy can be induced in a shorter time than by the method in which alcohol alone is administered. However, no report has been made on the application of this method to the screening for liver function protecting or improving agents.

[0011] A strong need exists for the development of pharmaceutical agents which effectively prevent or treat liver diseases and of health foods and drinks or animal feeds which can prevent or treat hepatopathy by daily intake.

SUMMARY OF THE INVENTION

[0012] The present invention provides a liver function protecting or improving agent, foods and drinks or feeds having liver function protecting or improving activity, additives for foods and drinks or for feeds having liver function protecting or improving activity, a method of screening for liver function protecting or improving agents, and a method of protecting or improving the animal liver function.

[0013] The present invention relates to the following (1) to (51).

[0014] (1) A liver function protecting or improving agent which comprises a plant of the family Saxifragaceae or an extract of the plant as an active ingredient.

[0015] (2) The liver function protecting or improving agent according to (1), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

[0016] (3) The liver function protecting or improving agent according to (2), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0017] (4) The liver function protecting or improving agent according to (1), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0018] (5) The liver function protecting or improving agent according to (4), wherein the plant belonging to the genus HVdrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0019] (6) The liver function protecting or improving agent according to any of (1) to (5), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

[0020] (7) The liver function protecting or improving agent according to any of (1) to (6), which is administered orally.

[0021] (8) The liver function protecting or improving agent according to any of (1) to (7), wherein the liver function is a function affected by alcohol.

[0022] (9) A food and drink which comprises a plant of the family Saxifragaceae or an extract of the plant.

[0023] (10) The food and drink according to (9), which is useful for the protection or improvement of liver function.

[0024] (11) The food and drink according to (10), wherein the liver function is a function affected by alcohol.

[0025] (12) The food and drink according to any of (9) to (11), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

[0026] (13) The food and drink according to (12), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0027] (14) The food and drink according to any of (9) to (11), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0028] (15) The food and drink according to (14), wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0029] (16) The food and drink according to any of (9) to (15), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

[0030] (17) A feed which comprises a plant of the family Saxifragaceae or an extract of the plant.

[0031] (18) The feed according to (17), which is useful for the protection or improvement of liver function.

[0032] (19) The feed according to (18), wherein the liver function is a function affected by alcohol.

[0033] (20) The feed according to any of (17) to (19), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

[0034] (21) The feed according to (20), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0035] (22) The feed according to any of (17) to (19), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0036] (23) The feed according to (22), wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0037] (24) The feed according to any of (17) to (23), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

[0038] (25) An additive for foods and drinks having liver function protecting or improving activity, which comprises a plant of the family Saxifragaceae or an extract of the plant.

[0039] (26) The additive for foods and drinks according to (25), wherein the liver function is a function affected by alcohol.

[0040] (27) The additive for foods and drinks according to (25) or (26), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraqa.

[0041] (28) The additive for foods and drinks according to (27), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0042] (29) The additive for foods and drinks according to (25) or (26), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0043] (30) The additive for foods and drinks according to (29), wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0044] (31) The additive for foods and drinks according to any of (25) to (30), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

[0045] (32) A feed additive having liver function protecting or improving activity, which comprises a plant of the family Saxifragaceae or an extract of the plant.

[0046] (33) The feed additive according to (32), wherein the liver function is a function affected by alcohol.

[0047] (34) The feed additive according to (32) or (33), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraqa.

[0048] (35) The feed additive according to (34), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0049] (36) The feed additive according to (32) or (33), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0050] (37) The feed additive according to (36), wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0051] (38) The feed additive according to any of (32) to (37), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

[0052] (39) A method of screening for liver function protecting or improving agents, which comprises administering an alcohol and then a lipopolysaccharide to an animal to raise the blood GPT or GOT level of the animal, administering a test substance to the animal, and estimating the activity of the test substance to lower the blood GPT or GOT level of the animal.

[0053] (40) The method according to (39), wherein the animal is a mammal.

[0054] (41) The method according to (39) or (40), wherein the lipopolysaccharide is derived from a microorganism belonging to the group of enteric bacteria.

[0055] (42) The method according to any of (39) to (41), wherein the liver function is a function affected by alcohol.

[0056] (43) A method of protecting or improving liver function in an animal, which comprises feeding the animal with the liver function protecting or improving agent according to any of (1) to (8) or the feed according to any of (17) to (24).

[0057] (44) The method according to (43), wherein the animal is selected from the group consisting of livestock, poultry and cultivated fish.

[0058] (45) A food and drink or feed for the protection or improvement of liver function which comprises a plant of the family Saxifragaceae or an extract of the plant as an active ingredient.

[0059] (46) The food and drink or feed according to (45), wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

[0060] (47) The food and drink or feed according to (46), wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

[0061] (48) The food and drink or feed according to (45), wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

[0062] (49) The food and drink or feed according to (48), wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

[0063] (50) The food and drink or feed according to any of (45) to (49), wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifraqaceae.

[0064] (51) The food and drink or feed according to any of (45) to (50), wherein the liver function is a function affected by alcohol.

DETAILED DESCRIPTION OF THE INVENTION

[0065] In the present invention, the term "protection of liver function" is intended to include the protection of liver function from various disorders and the prevention of liver function disorders. The term "improvement of liver function" is intended to include the improvement or cure of disordered liver function and the improvement or enhancement of liver function.

[0066] In the present invention, the term "plants of the family Saxifragaceae" means plants classified under the scientific name Saxifragaceae [Makino's Illustrated Flora in Color, 40th edition, Hokuryukan (June 10, 1984), Makino's New Illustrated Flora of Japan, 1st edition, Hokuryukan (May 10, 1983)].

[0067] The plants of the family Saxifragaceae include plants of the genera Astilbe, Rodgersia, Saxifraga, Tanakaea, Aceriphyllum, Boykinia, Chrysosplenium, Tiarell, Mitella, Parnassia, Philadelphus, Deutzia, Platycrater, Hydrangea, Schizophragma, Cardiandra, Deinanthe, Itea, Ribes and Kirengeshoma, and plants bred from these plants. Preferred are plants belonging to the genera Hydrangea and Saxifraga and those bred therefrom which have enhanced liver function protecting or improving activity.

[0068] Examples of the plants belonging to the genus Astilbe are *Astilbe microphylla* Knoll, *Astilbe thunbergii* Miq., *Astilbe odontophylla* Miq., *Astilbe japonica* Miq. and *Astilbe simplicifolia* Makino.

[0069] An example of the plants belonging to the genus Rodgersia is *Rodgersia podophylla* A. Gray.

[0070] Examples of the plants belonging to the genus Saxifraga are *Saxifraqa stolonifera* Meerb., *Saxifraga nipponica* Makino, *Saxifraga fortunei* Hook., *Saxifraga cortusaefolia* Sieb. et Zucc., *Saxifraga japonica* Boiss., *Saxifraga fusca* Maxim., *Saxifraga sendaica* Maxim. var. *laciniata* Nakai, *Saxifraga merkii* Fisch. var. *idsuroei* Engl., *Saxifraga laciniata* Nakai et Takeda, *Saxifraga bronchialalis* L., *Saxifraga cernua* L. and *Saxifraga sachalinensis* Fr. Schm. Preferred is *Saxifraha stolonifera* Meerb.

[0071] An example of the plants belonging to the genus Tanakaea is *Tanakaea radicans* Franch. et Sav.

[0072] An example of the plants belonging to the genus Aceriphyllum is *Aceriphyllum rossii* Engler.

[0073] Examples of the plants belonging to the genus Boykinia are *Boykinia lycoctonifolia* Engl. and *Boykinia tellimoides* Engl. et Irmsch. Examples of the plants belonging to the genus Chrysosplenium are *Chrysosplenium grayanum* Maxim., *Chrysosplenium stamineum* Franch, *Chrysosplenium japonicum* Makino, *Chrysosplenium flagelliferum* Fr. Schm., *Chrysosplenium macrostemon* Maxim. and *Chrysosplenium sphaerospermum* Maxim.

[0074] An example of the plants belonging to the genus Tiarella is *Tiarella polyphylla* Don.

[0075] Examples of the plants belonging to the genus Mitella are *Mitella japonica* Miq. and *Mitella pauciflora* Rosend.

[0076] Examples of the plants belonging to the genus Parnassia are *Parnassia palustris* L., *Parnassia alpicola* Makino, *Parnassia foliosa* Hook. f. et Thoms. var. nummularia Nakai.

[0077] An example of the plants belonging to the genus Philadelphus is *Philadelphus satsumi* Sieb.

[0078] Examples of the plants belonging to the genus Deutzia are *Deutzia crenata* Sieb. et Zucc., *Deutzia sieboldiana* Maxim., *Deutzia gracilis* Sieb. et. Zucc., *Deutzia maximowicziana* Makino, *Deutzia uniflora* Shirai and *Deutzia gracilis* Sieb. et Zucc. var. nagurai Makino.

[0079] An example of the plants belonging to the genus Platycrater is *Platycrater serrata* Makino.

[0080] Examples of the plants belonging to the genus Hydrangea are *Hydrangea macrophylla* Seringe, *Hydrangea macrophylla* Seringe var. otaksa Makino, *Hydrangea macrophylla* Seringe subsp. serrata Makino var. japonica Makino, *Hydrangea macrophylla* Seringe var. acuminata, *Hydrangea macrophylla* Seringe var. Thunbergii Makino, Hydrangeae Dulcis Folium, *Hydrangea scandens* Seringe, *Hydrangea hirta* Sieb. et Zucc., *Hydrangea involucrata* Sieb., *Hydrangea sikokiana* Maxim., *Hydrangea paniculata* Sieb., *Hydrangea petiolaris* Sieb. et Zucc., *Hydrangea macrophylla* Seringe subsp. *serrata* Makino var. *amoena* Makino, and *Hydrangea macrophylla* Seringe subsp. *serrata* Makino var. *angustata* Makino. Preferred are *Hydrangea macrophylla* Seringe var. Thunbergii Makino and Hydrangeae Dulcis Folium, and specifically preferred is Hydrangeae Dulcis Folium.

[0081] An example of the plants belonging to the genus Schizophragma is *Schizophragma hydrangeoides* Sieb. et Zucc.

[0082] An example of the plants belonging to the genus Cardiandra is *Cardiandra alternifolia* Sieb. et Zucc.

[0083] An example of the plants belonging to the genus Deinanthe is *Deinanthe bifida* Maxim.

[0084] An example of the plants belonging to the genus Itea is *Itea japonica* Oliver.

[0085] Examples of the plants belonging to the genus Ribes are *Ribes sinanense* F. Maekawa, *Ribes grossularia* L., *Ribes latifolium* Jancz., *Ribes rubrum* L., *Ribes japonicum* Maxim., *Ribes sachalinense* Nakai, *Ribes fasciculatum* Sieb. et Zucc., *Ribes alpinum* L. var. *japonicum* Maxim., and *Ribes ambiquum* Maxim.

[0086] An example of the plants belonging to the genus Kirengeshoma is *Kirengeshoma palmata* Yatabe.

[0087] The plants of the present invention include leaves, flowers, branches, stalks, fruits, roots, seeds, cultured cells or organs, callus, etc. of wild plants, cultivated plants or plants grown by culturing such as tissue culture, which are used as such or after being treated physically, chemically or biologically.

[0088] The physical or chemical treatment includes drying such as sun-drying, air-drying and freeze-drying, and disruption with a blender, a homogenizer, a ball mill, etc. The physically or chemically treated matters include dried matters, freeze-dried matters and disrupted matters. The biological treatment includes fermentation and the biologically treated matters include fermented matters.

[0089] The extracts of the plants include extracts obtained from the above-described plants by various methods of extraction. Examples of the methods of extraction are extraction with various solvents and supercritical fluid extraction. Extracts may further be treated by various methods of solid-liquid separation such as sedimentation, cake filtration, clear filtration, centrifugal filtration, centrifugal sedimentation, separation by compression and filter press, various concentration methods, various drying methods, methods of making various preparations such as granulation and pulverization, and various purification methods.

[0090] The purification methods include fractionation with a solvent, column chromatography and recrystallization. Specifically preferred is column chromatography using various carriers such as DIAION HP-20 (Mitsubishi Chemical Corporation) and Sephadex LH-20 (Pharmacia).

[0091] Examples of the concentration and drying methods are freeze-drying, natural drying, hot air-drying, air-drying, spray drying, drying under reduced pressure, sun-drying, vacuum drying, fluidized-bed drying, foam-bed drying, drum drying, ultrasonic drying and electromagnetic wave drying. Preferred are spray drying and freeze-drying.

[0092] In the step of extraction and treatment of an extract, an antioxidant, a preservative, etc. may be added.

[0093] As the solvent for extraction, any solvent which can extract a substance exhibiting liver function protecting or improving activity of the present invention can be used. Suitable solvents include aqueous media such as water, distilled water, deionized water, an aqueous solution of inorganic salt and buffer, monovalent alcohols such as methanol, ethanol, propanol and butanol, polyvalent alcohols such as propylene glycol and glycerol, and organic solvents such as hexane, toluene, petroleum ether, benzene, ethyl acetate, chloroform, dichloromethane, 1,1,2-trichloroethene, dimethyl sulfoxide and acetone. Preferred are aqueous media and alcohols.

[0094] Examples of the buffers are phosphate buffer and citrate buffer. Examples of the aqueous solutions of inorganic salts are those of sodium chloride, potassium chloride and calcium chloride.

[0095] Preferred alcohols are monovalent alcohols and a preferred monovalent alcohol is ethanol.

[0096] These solvents can be used alone or as a mixture. As the mixed solvent, water-containing alcohols are preferred. Water-containing monovalent alcohols are more preferred and water-containing ethanol is specifically preferred. The water content is preferably 70% or lower, more preferably 40% or lower.

[0097] As the solvent, supercritical fluid carbon dioxide may also be employed.

[0098] For extraction, the solvent is used in an amount of 0.1 to 10000 parts by weight, preferably 1 to 100 parts by weight for 1 part by weight of a plant. There is no specific restriction as to the temperature for extraction, but it is preferably 0 to 100° C., more preferably 20 to 90° C. There is no specific restriction as to the time for extraction, but it is preferably one minute to one week, more preferably 30 minutes to one day.

[0099] Specifically, extraction from the plants of the family Saxifragaceae is preferably carried out by extracting the above-described plants of the family Saxifragaceae, as such or after the physical, chemical or biological treatment, with an aqueous medium and then extracting the residues of the aqueous medium extracts of the plants with alcohol or water-containing alcohol.

[0100] There is no specific restriction as to the aqueous medium, but water, pure water and deionized water are preferred. The temperature for extraction with an aqueous medium, alcohol or water-containing alcohol is not specifically restricted, but is preferably 0 to 100° C., more preferably 20 to 90° C. The time for extraction is not specifically restricted, but is preferably one minute to one week, more preferably 30 minutes to one day. It is preferred to use the plants of the family Saxifragaceae after drying or fermenting treatment.

[0101] There is no specific restriction as to the apparatus to be employed for extraction. Preferred apparatuses include an apparatus designed for effective extraction, a stirrer, a reflux condenser, a Soxhlet extractor, a homogenizer, a shaker and a ultrasonic generator.

[0102] The liver function protecting or improving agent of the present invention comprises a plant of the family Saxifragaceae or an extract thereof prepared by the above-described method, and if necessary, may comprise one or more pharmaceutically acceptable carriers, and further, an active ingredient for another treatment.

[0103] The present pharmaceutical composition is prepared by mixing a plant of the family Saxifragaceae or an extract thereof with a carrier, as may be required, according to any of the methods well known in the field of pharmaceutics.

[0104] It is desirable to administer the composition by the route which is most effective for the treatment. Suitable administration routes include oral administration and non-oral administration such as intravenous administration, intraperitoneal administration or subcutaneous administration. Preferred is oral administration.

[0105] The composition is administered in the form of tablets, powders, granules, pills, suspensions, emulsions, infusa, capsules, syrups, injections, liquids, elixirs, extracts, tinctures, fluid extracts, etc.

[0106] Extracts, tinctures, fluid extracts, etc. suitable for oral administration can be prepared by extracting a plant of the family Saxifragaceae with water, ethanol or a mixture of water and ethanol, if necessary followed by concentration.

[0107] Liquid preparations suitable for oral administration such as syrups can be prepared using carriers such as water, sugars (e.g. sucrose, sorbitol and fructose), glycols (e.g. polyethylene glycol and propylene glycol), oils (e.g. sesame oil, olive oil and soybean oil), antiseptics (e.g. p-hydroxybenzoate), paraoxybenzoic acid derivatives (e.g. methyl paraoxybenzoate), preservatives (e.g. sodium benzoate) and flavors (e.g. strawberry flavor and peppermint).

[0108] Tablets, powders, granules, etc. suitable for oral administration can be prepared using sugars such as lactose, white sugar, glucose, sucrose, mannitol and sorbitol, starch such as potato starch, wheat starch and corn starch, inorganic substances such as calcium carbonate, calcium sulfate, sodium hydrogencarbonate and sodium chloride, excipients such as crystalline cellulose and plant powders (e.g. licorice powder and gentian powder), disintegrating agents such as starch, agar, gelatin powder, crystalline cellulose, carmellose sodium, carmellose calcium, calcium carbonate, sodium hydrogencarbonate and sodium alginate, lubricants such as magnesium stearate, talc, hydrogenated vegetable oil, macrogol and silicone oil, binders such as polyvinyl alcohol, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, carmellose, gelatin and starch paste, surfactants such as fatty acid ester, plasticizers such as glycerin, and the like.

[0109] Preparations appropriate for non-oral administration such as injections comprise, preferably, a sterilized aqueous agent containing an active compound which is isotonic to the recipient's blood. In the case of an injection, for example, an injectable solution is prepared using a carrier such as a salt solution, a glucose solution, or a mixture of a salt solution and a glucose solution.

[0110] The above-described antiseptics, preservatives, surfactants, etc. can also be employed in non-oral preparations.

[0111] The dose of the liver function protecting or improving agent of the present invention will vary depending on the administration route, the age and body weight of a patient and the symptom and degree of the disease to be treated, without specific restriction. For instance, when the agent is orally administered to an adult, it is suitable to administer the agent in an amount of 0.01 mg to 50 g, preferably 0.05 mg to 10 g in terms of dry weight of a plant of the family Saxifragaceae or an extract of the plant once to several times per day. In the case of non-oral administration such as intravenous administration, it is suitable to administer the agent in an amount of 0.001 mg to 50 g, preferably 0.01 mg to 10 g in terms of dry weight of a plant of the family Saxifragaceae or an extract of the plant once to several times per day. In the case of administration to an animal, the dose will vary depending on the age and kind of the animal and the symptom and degree of the disease, without specific restriction. It is generally suitable to administer the agent in an amount of 0.1 μg to 10 g, preferably 1 μg to 1 g per kg once to several times per day. In the case of non-oral administration such as intravenous administration, it is suitable to administer the agent in an amount of 0.01 μg to 10 g, preferably 1 μg to 1 g per kg once to several times per day. However, the dose may vary depending upon the above-mentioned conditions.

[0112] The foods and drinks or feeds having liver function protecting or improving activity which comprise a plant of the family Saxifragaceae or an extract of the plant include those prepared by adding to foods and drinks or feeds a plant of the family Saxifragaceae or an extract of the plant of the present invention in a process for producing ordinary foods and drinks or feeds. The foods and drinks or feeds of the present invention may be processed by molding and granulating methods. The molding and granulating methods include granulating methods such as fluidized bed granulation, stirring granulation, extrusion granulation, rolling granulation, air stream granulation, compression molding granulation, disruption granulation, spray granulation and blasting granulation, coating methods such as pan coating, fluidized bed coating and dry coating, plumping methods such as puff drying, excess steam method, foam mat method and microwave heating method, and extrusion methods using an extruding granulator and an extruder.

[0113] The foods and drinks or feeds for the protection or improvement of liver function comprising a plant of the family Saxifragaceae or an extract of the plant as an active ingredient include plants of the family Saxifragaceae and extracts of the plants themselves which can be used as foods

and drinks or feeds, and foods and drinks or feeds obtained by adding a plant of the family Saxifragaceae or an extract of the plant to foods and drinks, feeds or their starting materials.

[0114] There is no specific restriction as to the foods and drinks, feeds, or their starting materials to which a plant of the family Saxifragaceae or an extract of the plant is added. Useful foods and drinks, feeds, or their starting materials include both those which comprise a plant of the family Saxifragaceae or an extract of the plant and those which do not substantially comprise a plant of the family Saxifragaceae or an extract of the plant.

[0115] The liver function protecting or improving activity of the foods and drinks or feeds which comprise a plant of the family Saxifragaceae or an extract of the plant can be enhanced by adding a plant of the family Saxifragaceae or an extract of the plant thereto.

[0116] There is no specific restriction as to the amount of a plant of the family Saxifragaceae or an extract of the plant of the present invention to be added to foods and drinks or feeds, so long as it gives a content which enables foods and drinks or feeds to exhibit liver function protecting or improving activity. For instance, it is suitable to add a plant of the family Saxifragaceae or an extract of the plant in an amount of 0.001 to 100%, preferably 0.01 to 100%, more preferably 0.1-100%, in terms of dry weight.

[0117] Examples of the foods and drinks comprising a plant of the family Saxifragaceae or an extract of the plant are juice, soft drinks, soup, tea, dairy products (e.g. lactic acid bacteria beverages, fermented milk, ice cream, butter, cheese, yogurt, processed milk and skim milk), meat products (e.g. ham, sausage and hamburger), fish products, egg products (e.g. fried or steamed foods made of beaten eggs), confectionery (e.g. cookies, jelly, snacks and chewing gum), bread, noodles, pickles, smoked fish and meat, dry fish, preserved foods boiled down with soy and seasonings which comprise a plant of the family Saxifragaceae or an extract of the plant.

[0118] The foods and drinks may be in any of the forms such as a powder food, a sheet-shaped food, a bottled food, a canned food, a retort pouched food, a capsule food, a tablet food, a liquid food and a liquid nutrient food.

[0119] The foods and drinks of the present invention are used as health foods and drinks and functional foods and drinks for the protection or improvement of liver function.

[0120] There is no specific restriction as to the intake of foods and drinks of the present invention having liver function protecting or improving activity which comprise a plant of the family Saxifragaceae or an extract of the plant as an active ingredient. It is generally suitable to take a plant of the family Saxifragaceae or an extract of the plant in an amount of 0.1 to 50 g, preferably 0.5 to 10 g (dry weight) per adult per day for one day to one year, preferably 2 weeks to 3 months. This intake is merely a typical example and can be appropriately adjusted according to the recipient's condition, age, weight, etc.

[0121] The feeds of the present invention can be obtained, for example, by adding a plant of the family Saxifragaceae or an extract of the plant to feed materials.

[0122] The feeds of the present invention include any feeds having liver function protecting or improving activity on animals such as mammals, birds, reptiles, amphibians and fish. Examples of the feeds are feed for pets such as dogs, cats and mice, feed for livestock such as cows and pigs, feed for poultry such as hens and turkeys, and feed for cultivated fish such as sea breams and young yellowtails.

[0123] The feeds of the present invention can be prepared by appropriately mixing a plant of the family Saxifragaceae or an extract thereof with feed materials. The feed materials include grains, bran, vegetable oil cakes, animal feed materials, other feed materials and purified products.

[0124] Examples of the grains are milo, wheat, barley, oats, rye, brown rice, buckwheat, foxtail millet, broomcorn millet, Japanese millet, corn and soybean.

[0125] Examples of the bran are rice bran, defatted rice bran, wheat bran, wheat middlings, wheat germ, corn bran and corn germ.

[0126] Examples of the vegetable oil cakes are soybean oil cake, soybean flower, linseed oil cake, cottonseed oil cake, peanut oil cake, safflower oil cake, coconut oil cake, palm oil cake, sesame oil cake, sunflower oil cake, rapeseed oil cake, kapok oil cake and mustard seed oil cake.

[0127] Examples of the animal feed materials are fish meal (e.g. northern ocean meal, imported meal, whole meal and coastal meal), fish soluble, meat meal, meat and bone meal, blood powder, degradated hair, bone meal, treated by-products for livestock, feather meal, silkworm pupa, skim milk, casein and dry whey.

[0128] Examples of other feed materials are stalks and leaves of plants (e.g. alfalfa, hay cube, alfalfa leaf meal and powder of false acacia), processed industrial by-products of corn (e.g. corn gluten, meal, corn gluten feed and corn steep liquor), processed starch products (e.g. starch), sugar, fermentation industrial products (e.g. yeast, beer cake, malt root, alcohol cake and soy sauce cake), agricultural by-products (e.g. processed citrus fruit cake, tofu cake, coffee cake and cocoa cake) and others (e.g. cassava, broad bean, guar meal, seaweeds, krill, spirulina, chlorella and minerals).

[0129] Examples of the purified products are proteins (e.g. casein and albumin), amino acids, sugars (e.g. starch, cellulose, sucrose and glucose), minerals and vitamins.

[0130] There is no specific restriction as to the intake of the feeds of the present invention having liver function protecting or improving activity which comprise a plant of the family Saxifragaceae or an extract of the plant as an active ingredient. It is generally suitable to take a plant of the family Saxifragaceae or an extract of the plant in an amount of 0.1 mg to 50 g, preferably 0.5 mg to 10 g (dry weight) per kg per day for one day to one year, preferably 2 weeks to 3 months. This intake is merely a typical example and can be appropriately adjusted according to the kind, age, weight, etc. of an animal to be fed.

[0131] The additives for foods and drinks or feeds having liver function protecting or improving activity which comprise a plant of the family Saxifragaceae or an extract of the plant as an active ingredient comprise, as an active ingredient, a plant of the family Saxifragaceae or an extract thereof prepared according to the above-described method

and may comprise, if necessary, ordinary additives employed in foods and drinks or feeds, for example, additives listed in Food Additives Indication Pocket Book (Japan Food Additives Association, Jan. 6, 1997) such as sweeteners, coloring agents, preservatives, thickening stabilizers, antioxidants, color developing agents, bleaching agents, fungicides, gum bases, bitter agents, enzymes, wax, sour agents, seasonings, emulsifiers, nutrient supplements, additional materials for preparation, flavors and spice extracts. The carriers mentioned in the above description of pharmaceutical compositions may also be added.

[0132] Examples of the sweeteners are aspartame, licorice, stevia, xylose and *Momordica grosvenori*. Examples of the coloring agents are carotenoid pigment, turmeric pigment, flavonoid, caramel pigment, oriental gromurell pigment, spirulina pigment, chlorophyll, red sweet potato pigment, red Chinese yam pigment, perilla pigment and blueberry pigment.

[0133] Examples of the preservatives are sodium sulfite, benzoic acid and benzoates, extract of *Aralia cordata*, Japanese Styrax benzoin extract, Rumpet roman extract, sorbic acid and sorbates, and propionic acid and propionates. Examples of the thickening stabilizers are gums such as gum arabic and xanthane gum, alginic acid and alginates, chitin, chitosan, aloe extract, guar gum, hydroxypropyl cellulose, casein sodium, corn starch, carboxymethyl cellulose, gelatin, agar, dextrin, methyl cellulose, polyvinyl alcohol, microfibrous cellulose, microcrystalline cellulose, seaweed cellulose, sodium polyacrylate, sodium polyphosphate, carrageenan, yeast cell wall, extract of konjac, nata de coco and mannan.

[0134] Examples of the antioxidants are vitamin C, sodium ethylenediaminetetraacetate, calcium ethylenediaminetetraacetate, erythorbic acid, oryzanol, catechin, quercetin, clove extract, enzyme-treated rutin, apple extract, sesame oil extract, dibutylhydroxytoluene, fennel extract, horseradish extract, water dropwort extract, tea extract, Tempeh extract, extract of *Houttuynia cordata*, tocotrienol, tocopherols, rapeseed oil extract, green coffee extract, sunflower seed, ferulic acid, butylhydroxyanisole, blueberry leaf extract, propolis extract, hego-ginkgo leave extract, hesperetin, pepper extract, garden balsam extract, gallic acid, myrica extract, eucalyptus extract and rosemary extract.

[0135] An example of the color developing agent is sodium nitrite and an example of the bleaching agent is sodium sulfite.

[0136] An example of the fungicide is orthophenylphenol.

[0137] Examples of the gum bases are methyl acetylricinoleate, Japanese lacquer wax, ester gum, elemi resin, urucury wax, ozokerite, opopanax resin, kauri gum, carnauba wax, guaiacum resin, gutta katiau, gutta hangkang, guttapercha, glycerin fatty acid ester, spermaceti, copaiba balsam, copal resin, gum, rice bran wax, sugar cane wax, shellac, jelutong, sucrose fatty acid ester, sorba, sorbitan fatty acid ester, talc, calcium carbonate, dammar resin, chicle, chilte, tunu, low-molecular gum, paraffin wax, fir balsam, propylene glycol fatty acid ester, powdered pulp, powdered rice husks, jojoba wax, polyisobutylene, polybutene, microcrystalline wax, mastic, massaranduba chocolate, beeswax, and calcium phosphate.

[0138] Examples of the bitter agents are isoalpha bitter acid, caffeine, kawaratake extract, cinchona extract, Amur cork extract, gentian extract, spice extracts, enzyme-treated naringin, Jamaica quassia extract, theobromine, naringin, bitter ash extract, warmwood extract, isodonis extract, himematsutake extract, borapet, methyl thioadenosine, litchi extract, olive tea, sour orange extract, hop extract and mugwort extract.

[0139] Examples of the enzymes or enzyme sources are amylase, trypsin, rennet and lactic acid bacteria.

[0140] Examples of the wax are Japanese lacquer wax and vegetable wax. Examples of the sour agents are adipic acid, itaconic acid, citric acid and citrates, succinic acid and succinates, sodium acetate, tartaric acid and tartrates, carbon dioxide, lactic acid, phytic acid, fumaric acid, malic acid and phosphoric acid. Examples of the seasonings are amino acids such as asparagine, aspartic acid, glutamic acid, glutamine, alanine, isoleucine, glycine, serine, cystine, tyrosine, leucine and proline, nucleic acids such as sodium inosinate, sodium uridylate, sodium guanylate, sodium cytidylate, calcium ribonucleotide and sodium ribonucleotide, organic acids such as citric acid and succinic acid, potassium chloride, sodium solution of low salt content prepared from salt lake water, crude potassium chloride from sea water, whey salt, tripotassium phosphate, dipotassium hydrogenphosphate, potassium dihydrogenphosphate, disodium hydrogenphosphate, sodium dihydrogenphosphate, trisodium phosphate and chlorella extract.

[0141] Examples of the nutrient supplements are zinc salts, vitamin C, various amino acids, 5-adenylic acid, iron chloride, hesperidin, various kinds of burnt calcium, various kinds of unburnt calcium, dibenzoylthiamine, calcium hydroxide, calcium carbonate, thiamine hydrochloride, dunaliella carotene, tocopherol, nicotinic acid, carrot carotene, palm oil carotene, calcium pantothenate, vitamin A, hydroxyproline, calcium dihydrogenpyrophosphate, iron (II) pyrophosphate, iron (III) pyrophosphate, ferritin, heme iron, menaquinone, folic acid and riboflavin. Examples of the additional materials for preparation are processing aids such as acetone and ion exchange resin, extract of fig leaf, extract of rice straw ash, kaolin, glycerin fatty acid ester, mulberry extract, bone ash, perilla extract, ginger extract, various tannins, Phaffia color, grape seed extract and ethanol.

[0142] These additives can also be added to the above-described liver function protecting or improving agent and foods and drinks or feeds having liver function protecting or improving activity.

[0143] The term "liver function" as used herein means every function of the liver and there is no limit as to the definition of the term. Specific examples of the liver functions are those relating to blood and circulation such as storage of blood (adjustment of the amount of circulating blood, etc.), treatment of blood pigments (discharge of hemoglobin, etc.), formation of bile, enterohepatic circulation of bile pigments, and synthesis of plasma proteins (e.g. acute phase proteins, albumin, blood coagulation factors, steroid-binding proteins and other hormone-binding proteins), metabolic functions such as metabolism of nutrients and vitamins (e.g. glucose and other sugars, amino acids, lipids or fatty acids, cholesterol, lipoproteins, lipid-soluble vitamins and water-soluble vitamins), detoxification or

decomposition functions such as inactivation of various substances (e.g. toxins, steroids such as estrogen and androsterone, and other hormones) and immune functions ["Seirigaku Tenbo" (View of Physiology), 19th edition (Mar. 31, 2000), "Atarashii Rinsho Eiyogaku" (New Study of Clinical Nutrition), 3rd revision (May 20, 2000)]. These functions all suffer damage from an excessive intake of alcohol.

[0144] Described below is a method of screening for liver function protecting or improving agents, which comprises administering an alcohol and then a lipopolysaccharide to an animal to raise the blood GPT or GOT level of the animal, administering a test substance to the animal, and estimating the activity of the test substance to lower the blood GPT or GOT level of the animal.

[0145] There is no specific restriction as to the animal to be used for screening so far as it is an animal whose blood GPT or GOT level rises after administration of alcohol followed by administration of lipopolysaccharide. Suitable animals include mammals such as mice, rats, rabbits, dogs and cats.

[0146] Test substances for screening can be administered to animals by various administration routes such as feeding, oral administration, intraperitoneal administration, intravenous administration and intramuscular administration. Preferred are feeding and oral administration. Test substances can be administered to animals ad lib. or at regular intervals. For instance, a test substance is administered as a mixture with feed or as such once to three times a day for one hour to 360 days, preferably one day to 2 months, more preferably 3 to 15 days.

[0147] Alcohol can be administered to animals by oral administration, intravenous administration, or the like. Preferred is oral administration. Administration of alcohol may be carried out before, simultaneously with or after the administration of a test substance, but is preferably carried out after the administration of a test substance.

[0148] The amount of alcohol to be administered is 2 to 10 g/kg of an animal, preferably 3 to 5 g/kg. Administration is preferably carried out using a sound, etc. Alcohol is administered, for example, once to 360 times, preferably once to 10 times, for one hour to 360 days, preferably one day to 2 months, more preferably 3 days to 15 days. Then, a lipopolysaccharide is intravenously injected to the animal in an amount of 0.15 to 15 mg/kg, preferably 3 to 6 mg/kg, one to 24 hours, preferably 3 to 9 hours, specifically 6 hours after the administration of alcohol. The blood GPT or GOT level is determined 12 to 36 hours, preferably 20 to 28 hours later. This GPT or GOT level is compared with that obtained by administrating alcohol and then lipopolysaccharide to an animal without administration of a test substance. Screening for liver function protecting or improving agents can be carried out by selecting substances which lower the GPT or GOT level raised by the administration of alcohol and lipopolysaccharide.

[0149] Suitable lipopolysaccharides include those extracted from Gram-negative bacteria according to known methods. Examples of the Gram-negative bacteria are photosynthetic bacteria such as those belonging to the genus Rhodopseudomonas, bacteria belonging to the genus Pseudomonas, enteric bacteria such as those belonging to the genera Escherichia and Salmonella, lithotrophic bacteria such as those belonging to the genera Nitrobactor and Thiobacillus, and methane-forming bacteria such as those belonging to the genus Neisseria. Of these lipopolysaccharides, preferred are those derived from enteric bacteria, specifically those belonging to the genus Escherichia.

[0150] The screening method of the present invention enables advantageous screening for preventing or treating agents for alcoholic hepatopathy.

[0151] There is no specific restriction as to the method of determination of the GPT or GOT level so long as it can determine the GPT or GOT level. Determination of the GPT level can be carried out, for example, by determining pyruvic acid formed from 2-oxoglutamic acid and alanine. Determination of pyruvic acid can be carried out, for example, by measuring the decrease in the absorbance at 340 nm caused by the reduction of pyruvic acid in the presence of lactate dehydrogenase and NADH.

[0152] Determination of the GOT level can be carried out, for example, by determining oxaloacetic acid formed from aspartic acid and 2-oxoglutaric acid. Determination of oxaloacetic acid can be carried out, for example, by measuring the decrease in the absorbance at 340 nm caused by the reduction of oxaloacetic acid in the presence of malate dehydrogenase and NADH.

[0153] According to the present invention, liver function protecting or improving agents can be selected by selecting substances which significantly lower the blood GPT or GOT level in animals raised by administration of alcohol followed by administration of lipopolysaccharide.

[0154] Certain embodiments of the present invention are illustrated in the following examples. These examples are not to be construed as limiting the scope of the invention.

[0155] The following substances were used in the examples. Powder of Hydrangeae Dulcis Folium (Shihira Shoten), Powder of *Saxifraga stolonifera* Meerb. (Shihira Shoten), D-Galactosamine (Wako Pure Chemical Industries, Ltd.), Collagenase (Sigma Chemical Co., Ltd.), Acetaminophen (Wako Pure Chemical Industries, Ltd.), Penicillin (Gibco), Streptomycin (Gibco), DMSO (Wako Pure Chemical Industries, Ltd.), Insulin (Wako Pure Chemical Industries, Ltd.), Dexamethasone (Wako Pure Chemical Industries, Ltd.), TNF-α (Wako Pure Chemical Industries, Ltd.), Waymouth's MB752/1 (Gibco), FBS (Gibco), Pine-dex #3 (Matsutani Chemical Industry Co., Ltd.), Iron (III) pyrophosphate (Kokusan Chemical Works Co., Ltd.)

EXAMPLE 1

Production of a Freeze-Dried Powder of a Water Extract of Hydrangeae Dulcis Folium

[0156] Dry powder of Hydrangeae Dulcis Folium (1 kg, Shihira Shoten) was extracted twice with 10 l of distilled water at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 200 g of a freeze-dried powder of a water extract of Hydrangeae Dulcis Folium.

EXAMPLE 2

Production of a Freeze-Dried Powder of an Extract of Hydrangeae Dulcis Folium Extracted with a 60% Aqueous Solution of Ethanol

[0157] Dry powder of Hydrangeae Dulcis Folium (1 kg) was extracted twice with 10 l of a 60% aqueous solution of ethanol at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 200 g of a freeze-dried powder of an extract of Hydrangeae Dulcis Folium extracted with a 60% aqueous solution of ethanol.

EXAMPLE 3

Production of a Freeze-Dried Powder of an Acetone Extract of Hydrangeae Dulcis Folium

[0158] Dry powder of Hydrangeae Dulcis Folium (1 kg) was extracted twice with 10 l of acetone at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 100 g of a freeze-dried powder of an acetone extract of Hydrangeae Dulcis Folium.

EXAMPLE 4

Production of a Freeze-Dried Powder of a Hot Water Extract of Hydrangeae Dulcis Folium

[0159] Dry powder of Hydrangeae Dulcis Folium (1 kg) was extracted with 10 l of boiling distilled water for 30 minutes. The obtained extract was concentrated and freeze-dried to obtain 130 g of a freeze-dried powder of a hot water extract of Hydrangeae Dulcis Folium.

EXAMPLE 5

Production of a Freeze-Dried Powder of an Ethanol Extract of the Residue of a Water Extract of Hydrangeae Dulcis Folium

[0160] Dry powder of Hydrangeae Dulcis Folium (1 kg) was extracted with 20 l of distilled water at 40° C. with stirring until the absorbance of the 1/200 dilution at 313 nm became 0.15. The obtained extract was filtered and the filtrate was removed to obtain the extract residue. The residue was extracted with 20 l of 60% ethanol at 40° C. with stirring until the absorbance of the 1/200 dilution at 313 nm became 0.22. The obtained extract was concentrated and freeze-dried to obtain 70 g (yield based on dry leaves: 7%) of a freeze-dried powder of an ethanol extract of the residue of a water extract of Hydrangeae Dulcis Folium.

EXAMPLE 6

Production of a Freeze-Dried Powder of an Acetone Extract of the Residue of a Water Extract of Hydrangeae Dulcis Folium

[0161] Dry powder of Hydrangeae Dulcis Folium (1 kg) was extracted with 20 l of distilled water at 40° C. with stirring until the absorbance of the 1/200 dilution at 313 nm became 0.15. The obtained extract was filtered and the filtrate was removed to obtain the extract residue. The residue was extracted with 20 l of acetone at 40° C. with stirring until the absorbance of the 1/200 dilution at 313 nm became 0.22. The obtained extract was concentrated and freeze-dried to obtain 60 g (yield based on dry leaves: 6%) of a freeze-dried powder of an acetone extract of the residue of a water extract of Hydrangeae Dulcis Folium.

EXAMPLE 7

Production of a Feed Containing 3% Freeze-Dried Powder of Example 1

[0162] A feed having the following composition was prepared by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	37.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 1	3.0 wt %

EXAMPLE 8

Production of a Feed Containing 3% Freeze-Dried Powder of Example 2

[0163] A feed having the following composition was prepared by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	37.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 2	3.0 wt %

EXAMPLE 9

Production of a Feed Containing 3% Freeze-Dried Powder of Example 4

[0164] A feed having the following composition was prepared by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	37.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 4	3.0 wt %

EXAMPLE 10

Production of a Feed Containing 1% Freeze-Dried Powder of Example 5

[0165] A feed having the following composition was prepared by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	39.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 5	1.0 wt %

COMPARATIVE EXAMPLE 1

[0166] A feed having the following composition was prepared by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	40.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %

EXAMPLE 11

[0167] The freeze-dried powder produced in Example 1 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (5). Then, elution was carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (6). After further elution with 250 ml of 100% methanol, elution was carried out with 250 ml of 100% acetone. The resulting eluate was concentrated to dryness to obtain concentrate (8). Then, elution was carried out with 250 ml of 100% acetone and the resulting eluate was concentrated to dryness to obtain concentrate (9).

EXAMPLE 12

[0168] The freeze-dried powder produced in Example 4 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (5). Then, elution was carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (6). After further elution with 250 ml of 100% methanol, elution was carried out with 250 ml of 100% acetone. The resulting eluate was concentrated to dryness to obtain concentrate (8). Then, elution was carried out with 250 ml of 100% acetone and the resulting eluate was concentrated to dryness to obtain concentrate (9).

EXAMPLE 13

[0169] The freeze-dried powder produced in Example 2 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (5). Then, elution was carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (6). Elution was further carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (7). Subsequently, elution was carried out with 250 ml of 100% acetone, followed by further elution with 250 ml of 100% acetone. The resulting eluate was concentrated to dryness to obtain concentrate (9).

EXAMPLE 14

[0170] The freeze-dried powder produced in Example 5 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (5). Then, elution was carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (6). Elution was further carried out with 250 ml of 100% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (7). Subsequently, elution was carried out with 250 ml of 100% acetone, followed by further elution with 250 ml of 100% acetone. The resulting eluate was concentrated to dryness to obtain concentrate (9).

EXAMPLE 15

[0171] The freeze-dried powder of the acetone extract produced in Example 3 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml). The column was washed successively with two 250 ml

portions of 33% methanol, two 250 ml portions of 66% methanol and further two 250 ml portions of 66% methanol. After washing with 250 ml of 100% methanol, elution was carried out with 250 ml of 100% acetone and the resulting eluate was concentrated to dryness to obtain concentrate (8).

EXAMPLE 16

Inhibiting Activity of Extracts of Hydrangeae Dulcis Folium on D-Galactosamine-Induced Rat Hepatopathy

[0172] Groups of male Wistar white rats (150±20 g, Japan SLC) were kept for at least 3 days under fixed conditions (temperature: 24±2° C., humidity: 60±5%, dark and bright interval: 12 hours) for adaptation, and then fed respectively with the feeds produced in Examples 7-10 (test groups) and the feed produced in Comparative Example 1 (control group) for 15 days. On the 14th day, 350 mg/kg of D-galactosamine (dissolved in physiological saline at a concentration of 35 mg/ml, pH 7.1) was intraperitoneally administered to each rat. Twenty-two hours after the administration of D-galactosamine, each of the rats was subjected to laparotomy under anesthesia with Nembutal and blood was sampled.

[0173] The thus obtained blood samples were subjected to measurement of blood GPT activity as an indication of liver function in the following manner. The sampled blood was coagulated and separated by centrifugation to obtain a serum. The GPT level in the obtained serum was measured using Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd.). The GPT activity of each test group was calculated as the relative value (%) based on the value of control group expressed as 100%. The value is expressed in terms of average value ±standard error and the statistical test of significance was carried out by T-test.

[0174] The results are shown in Table 1.

TABLE 1

Feed	GPT activity (%)	Test of significance
Feed of Example 7	49.8 ± 21.6	p = 0.0265
Feed of Example 9	40.7 ± 9.7	p = 0.0000212
Feed of Example 8	30.5 ± 14.6	p = 0.0079
Feed of Example 10	14.3 ± 6.9	p = 0.000373

[0175] When the feeds of Examples 7-10 were administered, the serum GPT activity which is an indication of liver function disorder was as low as 14.3 to 49.8% of that obtained with the feed of Comparative Example 1. This indicates that hepatopathy was inhibited. In the case of the feed of Example 10, the GPT activity was 14.3%, which indicates that the ethanol extract of the residue of a water extract of Hydrangeae Dulcis Folium produced in Example 5 has a strong hepatopathy inhibiting activity.

[0176] During 15 days of the feeding, there was no difference among the groups in weight increase, and no abnormality was recognized in appearance or action.

EXAMPLE 17

Inhibiting Activity of a Fraction of an Acetone Extract of Hydrangeae Dulcis Folium on Acetaminophen-induced Disorder of Primary Cultured Hepatocytes

[0177] Hepatocytes of a rat were separated according to the collagenase perfusion method [Seglen, P. O., Methods in Cell Biology, 13, 29 (1976)]. That is, a male SD rat weighing ca. 130 g was subjected to laparotomy under anesthesia, and 400 ml of a preperfusion liquid [a solution prepared by dissolving 9.5 g of Hanks' Balanced Salt Solution (Gibco), 2.38 g of HEPES (Nacalai Tesque, Inc.), 0.19 g of EGTA (Sigma Chemical Co., Ltd.) and 0.35 g of $NaHCO_3$ (Kishida Chemical Co., Ltd.) in 1 l of water, pH 7.2] maintained at 37° C. was perfused through a portal vein at a flow rate of 30 ml/minute. Then, 200 ml of a collagenase solution [a solution prepared by dissolving 9.8 g of Hanks' solution Nissui (1) (Nissui Pharmaceutical Co., Ltd.), 2.38 g of HEPES (Nacalai Tesque, Inc.), 0.35 g of $NaHCO_3$ (Kishida Chemical Co., Ltd.), 0.56 g of $CaCl_2$ (Kishida Chemical Co., Ltd.), 0.02 g of trypsin inhibitor (Sigma Chemical Co., Ltd.) and 0.5 g of collagenase (Sigma Chemical Co., Ltd.) in 1 l of water, pH 7.5] maintained at 37° C. was perfused. After being digested, the liver was put in a petri dish, and 20 ml of S-MEM medium (Gibco) was added thereto, followed by mincing with a surgical knife. The hepatocytes were dispersed by pipetting with a 10-ml Komagome pipette and then filtered through a gauze patch and a cell filter (Ikemoto Scientific Technology Co., Ltd.) to obtain a hepatocyte dispersion. The obtained hepatocytes contained not only liver parenchymal cells, but also non-parenchymal cells such as endothelial cells, Kupffer cells, Ito cells and star cells, and liver parenchymal cells alone were purified by centrifugation. That is, the obtained hepatocyte dispersion was centrifuged at a low speed (50× g) under cooling for one minute, and the precipitated liver parenchymal cells were recovered. This operation was repeated three times to separate and recover the liver parenchymal cells of high purity.

[0178] The recovered hepatocytes were suspended in the following basal medium at a density of 1.2×10^6 cells/ml, and wells of a matri gel-coated 6-well plate were seeded with 1.4 ml of the resulting suspension, followed by primary culture under the following culture conditions.

[0179] The medium was prepared by adding fetal bovine serum (FBS, 10%), penicillin (50 U/ml), streptomycin (50 μg/ml), insulin (10^{-8} M) and dexamethasone (10^{-6} M) to Waymouth's MB752/1 medium (Gibco) (hereinafter sometimes referred to as a basal medium).

[0180] Culturing was carried out in a CO_2 incubator (5% CO_2, 95% air) at 37° C. Four hours after the seeding, the basal medium was removed and the wells were washed with PBS. Then, 0.7 ml of a fresh basal medium was pipetted into each well, followed by further culturing.

[0181] Concentrate (8) obtained in Example 15 was dissolved in dimethyl sulfoxide (hereinafter abbreviated as DMSO) in a concentration of 10 mg/ml, and the resulting solution was diluted 50-fold with the above basal medium to prepare a test solution having a concentration of 200 μg/ml.

[0182] Twenty-four hours after the seeding of cells, the test solution was added to wells in an amount of 140 μl per

well (final concentration: 20 μg/ml), and one hour later, 50 mM acetaminophen (a hepatopathy inducer) dissolved in the medium was added thereto in an amount of 560 μl per well (final concentration: 20 mM) (test group).

[0183] Separately, 24 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and one hour later, 50 mM acetaminophen (a hepatopathy inducer) was added thereto in an amount of 560 μl per well (final concentration: 20 mM) (control group 1).

[0184] Further, 24 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and one hour later, the medium was added thereto in an amount of 560 μl per well (control group 2).

[0185] Forty-eight hours after the addition of acetaminophen or medium, the number of cells of each group was estimated by means of absorbance by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay. That is, the basal medium was removed from each well and 1.4 ml of Waymouth's MB752/1 medium containing 10% PBS containing MTT (10 mg/ml) was pippetted therein. After incubation in a CO_2 incubator at 37° C. for one hour, 4.2 ml of DMSO was added thereto. After vigorous stirring, the absorbance of each group was measured at 570 nm using a microplate reader (Bio Rad, Model 3550). Evaluation was made in duplicate. The hepatocyte disorder inhibiting rate was calculated by the following equation.

$$\text{Hepatocyte disorder inhibiting rate } (\%)=[(A-B)/(C-B)]\times 100 \quad \text{[Equation 1]}$$

[0186] A: Absorbance of test group

[0187] B: Absorbance of control group 1

[0188] C: Absorbance of control group 2

[0189] The result is shown in Table 2.

TABLE 2

Hepatocyte disorder inhibiting rate (%)	42.2

[0190] As shown in Table 2, concentrate (8) obtained in Example 15 inhibited the hepatocyte disorder caused by acetaminophen by 42.2%.

EXAMPLE 18

Inhibiting Activity of Fractions of Extracts of Hydrangeae Dulcis Folium on D-Galactosamine-Induced Disorder of Primary Cultured Hepatocytes

[0191] Each of concentrates (3), (4), (5), (6), (8) and (9) obtained in Examples 11 and 12 and concentrates (3), (4), (5), (6) and (7) obtained in Examples 13 and 14 was dissolved in DMSO in a concentration of 10 mg/ml. Each of the resulting solutions was diluted 50-fold with the basal medium to prepare a diluted solution (concentration: 200 μg/ml). Rat hepatocytes were seeded and primary-cultured in the same manner as in Example 17. Two hours after the seeding of cells, the medium was removed and the wells were washed with PBS. Then, 0.7 ml of a fresh medium was pippetted into each well, and 140 μl of the diluted solution was added thereto (final concentration: 20 μg/ml). Two hours later, 560 μl of 50 mM D-galactosamine (a hepatopathy inducer) dissolved in the medium was added thereto (final concentration: 20 mM) (test group).

[0192] Separately, 2 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and 2 hours later, 50 mM D-galactosamine (a hepatopathy inducer) dissolved in the medium was added thereto in an amount of 560 μl per well (final concentration: 20 mM) (control group 1).

[0193] Further, 2 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and 2 hours later, the medium was added thereto in an amount of 560 μl per well (control group 2).

[0194] Forty-eight hours after the addition of D-galactosamine or medium, the number of cells of each group was estimated by means of absorbance by MTT assay. That is, the basal medium was removed from each well and 1.4 ml of Waymouth's MB752/1 medium containing 10% PBS containing MTT (10 mg/ml) was pippetted therein. After incubation in a CO_2 incubator at 37° C. for one hour, 4.2 ml of DMSO was added thereto. After vigorous stirring, the absorbance of each group was measured at 570 nm using a microplate reader (Bio Rad, Model 3550). Evaluation was made in duplicate. The hepatocyte disorder inhibiting rate was calculated by the above equation 1.

[0195] The results are shown in Table 3.

TABLE 3

Fraction of extract of Hydrangeae Dulcis Folium	Hepatocyte disorder inhibiting rate (%)
Concentrate (3) of Example 12	59.1
Concentrate (4) of Example 12	73.0
Concentrate (5) of Example 12	67.7
Concentrate (6) of Example 12	45.4
Concentrate (8) of Example 12	31.8
Concentrate (9) of Example 12	44.9
Concentrate (3) of Example 11	56.2
Concentrate (4) of Example 11	55.8
Concentrate (5) of Example 11	55.9
Concentrate (6) of Example 11	54.9
Concentrate (8) of Example 11	40.2
Concentrate (9) of Example 11	47.0
Concentrate (3) of Example 13	76.0
Concentrate (4) of Example 13	97.4
Concentrate (5) of Example 13	66.8
Concentrate (6) of Example 13	89.1
Concentrate (7) of Example 13	46.1
Concentrate (3) of Example 14	65.2
Concentrate (4) of Example 14	79.9
Concentrate (5) of Example 14	34.0
Concentrate (6) of Example 14	45.8
Concentrate (7) of Example 14	33.2

[0196] As shown in Table 3, the fractions of extracts of Hydrangeae Dulcis Folium inhibited the hepatocyte disorder caused by D-galactosamine by 31.8 to 97.4%.

EXAMPLE 19

Inhibiting Activity of Fractions of Extracts of Hydrangeae Dulcis Folium on D-Galactosamine/TNF-α-induced Disorder of Primary Cultured Hepatocytes

[0197] Each of concentrates (9) obtained in Examples 12, 13 and 14 was dissolved in DMSO in a concentration of 10

mg/ml, and the resulting solution was diluted 50-fold with the basal medium to prepare a diluted solution (concentration: 500 μg/ml).

[0198] Rat hepatocytes were seeded and primary-cultured in the same manner as in Example 17. Two hours after the seeding of cells, the medium was removed and the wells were washed with PBS. Then, 0.7 ml of a fresh medium was pippetted into each well, and 140 μl of the diluted solution was added thereto (final concentration: 20 μg/ml). Two hours later, a mixture of 2.5 mM D-galactosamine dissolved in the medium and 2.5 ng/ml TNF-α (hereinafter referred to as GT solution) was added thereto in an amount of 560 μl (final concentration: D-galactosamine, 1 mM; TNF-α, 1 ng/ml) (test group).

[0199] Separately, 2 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and 2 hours later, GT solution (a hepatopathy inducer) was added thereto in an amount of 560 μl per well (final concentration, D-galactosamine, 1 mM; TNF-α, 1 ng/ml) (control group 1).

[0200] Further, 2 hours after the seeding of cells, a basal medium containing 2% DMSO was added to wells in an amount of 140 μl per well, and 2 hours later, the medium was added thereto in an amount of 560 μl per well (control group 2).

[0201] Forty-eight hours after the addition of GT solution or medium, the number of cells of each group was estimated by means of absorbance by MTT assay. That is, the basal medium was removed from each well and 1.4 ml of Waymouth's MB752/1 medium containing 10% PBS containing MTT (10 mg/ml) was pippetted therein. After incubation in a CO_2 incubator at 37° C. for one hour, 4.2 ml of DMSO was added thereto. After vigorous stirring, the absorbance of each group was measured at 570 nm using a microplate reader (Bio Rad, Model 3550). Evaluation was made in duplicate. The hepatocyte disorder inhibiting rate was calculated by the above equation 1.

[0202] The results are shown in Table 4.

TABLE 4

Fraction of extract of Hydrangeae Dulcis Folium	Hepatocyte disorder inhibiting rate (%)
Concentrate (9) of Example 12	34.0
Concentrate (9) of Example 13	27.4
Concentrate (9) of Example 14	17.2

[0203] As shown in Table 4, the fractions of extracts of Hydrangeae Dulcis Folium inhibited the hepatocyte disorder caused by a mixture of D-galactosamine and TNF-α by 17.2 to 34.0%.

EXAMPLE 20

[0204] A feed having the following composition was prepared by mixing the ingredients.

CE-2 (Clea Japan, Inc)	99 wt %
Powder produced in Example 5	1 wt %

EXAMPLE 21

[0205] A feed having the following composition was prepared by mixing the ingredients.

CE-2 (Clea Japan, Inc)	99 wt %
Powder produced in Example 3	1 wt %

COMPARATIVE EXAMPLE 2

[0206] A feed having the following composition was prepared by mixing the ingredients.

CE-2 (Clea Japan, Inc)	99 wt %
Pine-dex #3 (Matsutani Chemical Industry Co., Ltd.)	1 wt %

EXAMPLE 22

Inhibiting Activity of an Ethanol Extract of the Residue of a Water Extract of Hydrangeae Dulcis Folium on Alcohol/LPS-Induced Rat Hepatopathy

[0207] roups of female SD white rats (ca. 200 g, Japan SLC) were kept for at least 3 days under fixed conditions (temperature: 24±2° C., humidity: 60±5%, dark and bright interval: 12 hours) for adaptation and then fed respectively with the feed produced in Example 20 (test group) and the feed produced in Comparative Example 2 (control group) for 15 days. During 15 days of the feeding, there was no difference between the two groups in weight increase, and no abnormality was recognized in appearance or action. On the 14th day, 4 g/kg of alcohol [prepared as a 40% (v/v) ethanol solution] was orally administered to the rats of both groups using a sound. Six hours later, 5 mg/kg of a solution prepared by dissolving LPS (derived from *E. coli*, Sigma Chemical Co., Ltd.) in physiological saline at a concentration of 5 mg/ml was intravenously injected. Twenty-four hours after the injection, each of the rats was subjected to laparotomy under anesthesia with Nembutal and blood was sampled. The test was carried out on the two groups each consisting of 6 rats.

[0208] The thus obtained blood samples were subjected to measurement of blood GPT and GOT activities as indications of liver function in the following manner. The sampled blood was coagulated and separated by centrifugation to obtain a serum. The GPT and GOT levels in the obtained serum were measured using Fuji Drychem System 3500 (Fuji Photo Film Co., Ltd.). The GPT and GOT activities of test group were calculated as the relative values based on the values of control group expressed as 100%. The values are

expressed in terms of average value ±standard error and the statistical test of significance was carried out by T-test.

[0209] The results are shown in Table 5.

TABLE 5

	Relative value based on control group (%)	Test of significance
GPT activity	10.8 ± 1.6	p = 0.002
GOT activity	7.7 ± 1.1	p = 0.003

[0210] In the test group to which the extract of Hydrangeae Dulcis Folium was administered, the serum GPT and GOT activities which are indications of liver function disorder were as low as 10.8% and 7.7% of those of the control group. This indicates that hepatopathy was inhibited. By the test of significance, the difference was recognized as significant ($p<0.01$).

EXAMPLE 23

Inhibiting Activity of an Acetone Extract of Hydrangeae Dulcis Folium on D-Galactosamine-Induced Rat Hepatopathy

[0211] Groups of male SD white rats (150±20 g, Japan SLC) were kept for at least 3 days under fixed conditions (temperature: 24±2° C., humidity: 60±5%, dark and bright interval: 12 hours) for adaptation, and then fed respectively with the feed produced in Example 21 (test group) and the feed produced in Comparative Example 2 (control group) for 4 days. The rats of both groups were fasted for 18 hours and then 400 mg/kg of D-galactosamine (dissolved in physiological saline at a concentration of 40 mg/ml) was intraperitoneally administered to each rat. Twenty-two hours after the administration of D-galactosamine, each of the rats was subjected to laparotomy under anesthesia with Nembutal and blood was sampled.

[0212] The thus obtained blood samples were subjected to measurement of blood GPT activity as an indication of liver function in the following manner. The sampled blood was coagulated and separated by centrifugation to obtain a serum. The GPT level in the obtained serum was measured using Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd.). The GPT activity of test group was calculated as the relative value (%) based on the value of control group expressed as 100%. The value is expressed in terms of average value ±standard error, and the statistical test of significance was carried out by T-test.

[0213] The result is shown in Table 6.

TABLE 6

Feed	Extract concentration	GPT activity (%)	Test of significance
Example 21	1%	56.4 ± 9.9	p = 0.0069

[0214] When the feed of Example 21 was administered, the serum GPT activity which is an indication of liver function disorder was as low as 56.4% of that obtained with the feed of Comparative Example 2. This indicates that hepatopathy was inhibited.

[0215] During the feeding period, there was no difference between the two groups in weight increase, and no abnormality was recognized in appearance or action.

EXAMPLE 24

Production of a Freeze-Dried Powder of a Water Extract of *Saxifraga stolonifera* Meerb.

[0216] Dry powder of *Saxifraga stolonifera* Meerb. (1 kg, Shihira Shoten) was extracted twice with 10 1 of distilled water at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 150 g of a freeze-dried powder of a water extract of *Saxifraga stolonifera* Meerb.

EXAMPLE 25

Production of a Freeze-Dried Powder of an Extract of *Saxifraga stolonifera* Meerb. Extracted with a 60% Aqueous Solution of Ethanol

[0217] Dry powder of *Saxifraga stolonifera* Meerb. (1 kg) was extracted twice with 10 l of a 60% aqueous solution of ethanol at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 188 g of a freeze-dried powder of an extract of *Saxifraga stolonifera* Meerb. extracted with a 60% aqueous solution of ethanol.

EXAMPLE 26

Production of a Freeze-Dried Powder of an Acetone Extract of *Saxifraga stolonifera* Meerb.

[0218] Dry powder of *Saxifraga stolonifera* Meerb. (1 kg) was extracted twice with 10 1 of acetone at room temperature with stirring for one hour. The obtained extract was concentrated and freeze-dried to obtain 164 g of a freeze-dried powder of an acetone extract of *Saxifraga stolonifera* Meerb.

EXAMPLE 27

Production of a Freeze-Dried Powder of a Hot Water Extract of Saxifraga stolonifera Meerb.

[0219] Dry powder of Saxifraga stolonifera Meerb. (1 kg) was extracted with 10 1 of boiling distilled water for 30 minutes. The obtained extract was concentrated and freeze-dried to obtain 100 g of freeze-dried powder of a hot waster extract of *Saxifraqa stolonifera* Meerb.

EXAMPLE 28

[0220] The freeze-dried powder produced in Example 24 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol, and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (5).

EXAMPLE 29

[0221] The freeze-dried powder produced in Example 25 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. Elution was further carried out with 250 ml of 33% methanol, and the eluate was concentrated to dryness to obtain concentrate (3). Then, elution was carried out with 250 ml of 66% methanol, and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol, and the resulting eluate was concentrated to dryness to obtain concentrate (5). Then, elution was carried out with 250 ml of 100% methanol, and the resulting eluate was concentrated to dryness to obtain concentrate (6).

EXAMPLE 30

[0222] The freeze-dried powder produced in Example 26 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with 250 ml of 33% methanol. The resulting eluate was concentrated to dryness to obtain concentrate (2). After further elution with 250 ml of 33% methanol, elution was carried out with 250 ml of 66% methanol. The resulting eluate was concentrated to dryness to obtain concentrate (4).

EXAMPLE 31

[0223] The freeze-dried powder produced in Example 27 (5 g) was suspended in 500 ml of water. The resulting suspension was passed through a column packed with a hydrophobic adsorbent resin (Mitsubishi Chemical Corporation, DIAION HP-20, void volume: 100 ml), followed by elution with two 250 ml portions of 33% methanol. Then, elution was carried out with 250 ml of 66% methanol and the resulting eluate was concentrated to dryness to obtain concentrate (4). Elution was further carried out with 250 ml of 66% methanol, and the resulting eluate was concentrated to dryness to obtain concentrate (5).

EXAMPLE 32

[0224] A feed having the following composition was prepared by mixing the ingredients.

CE-2 (Clea Japan, Inc.)	99.0 wt %
Powder produced in Example 24	1.0 wt %

EXAMPLE 33

[0225] A feed having the following composition was prepared by mixing the ingredients.

CE-2 (Clea Japan, Inc.)	99.0 wt %
Powder produced in Example 25	1.0 wt %

EXAMPLE 34

Inhibiting Activity of Extracts of *Saxifraga stolonifera* Meerb. [Water Extract (Example 24) and 60% Ethanol Extract (Example 25)] on D-Galactosamine-Induced Rat Hepatopathy

[0226] Groups of male SD white rats (150±20 g, Japan SLC) were kept for at least 3 days under fixed conditions (temperature: 24±2° C., humidity: 60±5%, dark and bright interval: 12 hours) for adaptation, and then fed respectively with the feeds produced in Examples 32 and 33 (test groups) and the feed produced in Comparative Example 2 (control group) for 3 days. After the rats were fasted for 18 hours, 400 mg/kg of D-galactosamine (dissolved in physiological saline at a concentration of 40 mg/ml) was intraperitoneally administered to each rat. Then, the rats were fed with the above respective feeds for one day, and each of the rats was subjected to laparotomy under anesthesia with Nembutal and blood was sampled.

[0227] The thus obtained blood samples were subjected to measurement of blood GPT activity as an indication of liver function in the following manner. The sampled blood was coagulated and separated by centrifugation to obtain a serum. The GPT level in the obtained serum was measured using Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd.). The GPT activity of each test group was calculated as the relative value (%) based on the value of control group expressed as 100%. The value is expressed in terms of average value ±standard error and the statistical test of significance was carried out by T-test.

[0228] The results are shown in Table 7.

TABLE 7

Feed	Extract Concentration	GPT activity (%)	Test of significance
Example 32	1%	35.7 ± 6.1	p = 0.0224
Example 33	1%	29.9 ± 8.0	p = 0.0034

[0229] When the feeds of Examples 32 and 33 were administered, the serum GPT activity which is an indication of liver function disorder was as low as 35.7% and 29.9%, respectively, of that obtained with the feed of Comparative Example 2. This indicates that hepatopathy was inhibited. The difference was recognized as significant by the test of significance.

[0230] During the feeding period, there was no difference among the groups in weight increase, and no abnormality was recognized in appearance or action.

EXAMPLE 35

Inhibiting Activity of Fractions of an Acetone Extract of *Saxifraga stolonifera* Meerb. on Acetaminophen-Induced Disorder of Primary Cultured Hepatocytes

[0231] Experiment was carried out in the same manner as in Example 17 using concentrates (2) and (4) obtained in Example 30.

[0232] The results are shown in Table 8.

TABLE 8

Concentrate of Example 30	Hepatocyte disorder inhibiting rate (%)
(2)	46.7
(4)	46.4

[0233] As shown in Table 8, the fractions of the acetone extract of *Saxifraga stolonifera* Meerb. inhibited the hepatocyte disorder caused by acetaminophen by 46.7% and 46.4%, respectively.

EXAMPLE 36

Inhibiting Activity of Fractions of Extracts of *Saxifraga stolonifera* Meerb. on D-Galactosamine-Induced Disorder of Primary Cultured Hepatocytes

[0234] Experiment was carried out in the same manner as in Example 18 using the concentrates obtained in Examples 28, 29, 30 and 31.

[0235] The results are shown in Table 9.

TABLE 9

Concentrate	Hepatocyte disorder inhibiting rate (%)
Concentrate (4) of Ex. 31	49.1
Concentrate (5) of Ex. 31	37.7
Concentrate (3) of Ex. 28	83.8
Concentrate (4) of Ex. 28	69.0
Concentrate (5) of Ex. 28	111.1
Concentrate (3) of Ex. 29	36.1
Concentrate (4) of Ex. 29	65.1
Concentrate (5) of Ex. 29	56.7
Concentrate (6) of Ex. 29	66.6
Concentrate (5) of Ex. 30	56.0
Concentrate (4) of Ex. 30	66.5

[0236] As shown in Table 9, the fractions of extracts of *Saxifraga stolonifera* Meerb. inhibited the hepatocyte disorder caused by D-galactosamine by 36.1 to 111.1%.

EXAMPLE 37

Inhibiting Activity of Fractions of Extracts of *Saxifraga stolonifera* Meerb. on D-Galactosamine/TNF-α-Induced Disorder of Primary Cultured Hepatocytes

[0237] Experiment was carried out in the same manner as in Example 19 using the concentrates obtained in Examples 28, 29 and 31.

[0238] The results are shown in Table 10.

TABLE 10

Concentrate	Hepatocyte disorder inhibiting rate (%)
Concentrate (4) of Ex. 31	30.3
Concentrate (5) of Ex. 31	17.0
Concentrate (3) of Ex. 28	32.4
Concentrate (4) of Ex. 28	53.6
Concentrate (5) of Ex. 28	59.7
Concentrate (4) of Ex. 29	58.5
Concentrate (5) of Ex. 29	90.6
Concentrate (6) of Ex. 29	64.8

[0239] As shown in Table 10, the fractions of extracts of *Saxifraga stolonifera* Meerb. inhibited the hepatocyte disorder caused by a mixture of D-galactosamine and TNF-α by 17.0 to 90.6%.

EXAMPLE 38

Production of a Preparation Containing an Ethanol Extract of the Residue of a Water Extract of Hydrangeae Dulcis Folium

[0240] A liver function protecting or improving agent having the following composition was produced by mixing the ingredients.

Ethanol extract of the residue of a water extract of Hydrangeae Dulcis Folium produced in Example 5	49 g
Pine-dex #3	49 g
Iron (III) pyrophosphate (iron source)	0.1 g
Phoscal EFC (calcium source, Nikko Fine Products)	1 g
Vitamin Mix (Merck & Co., Inc.)	1 g

EXAMPLE 39

[0241] The liver function protecting or improving agent produced in Example 38 (20 g) was dispersed into 180 ml of water to produce a liver function protecting or improving drink.

EXAMPLE 40

Production of Cookies Containing an Ethanol Extract of the Residue of a Water Extract of Hydrangeae Dulcis Folium

[0242] Cookies (30 pieces) were prepared from the following ingredients according to a conventional method.

Soft flour	100 g
Starch	74 g
Water	14 g
Ethanol extract of the residue of a water extract of Hydrangeae Dulcis Folium produced in Example 5	30 g
Baking powder	2 Tsp.
Salt	2 Tsp.
Egg	1
Butter	80 g
Milk	2 Tbsp.

EXAMPLE 41

Production of a Feed Containing 1% Freeze-Dried Powder of Example 24

[0243] A feed having the following composition was produced by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	39.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 24	1.0 wt %

EXAMPLE 42

Production of a Feed Containing 1% Freeze-Dried Powder of Example 25

[0244] A feed having the following composition was produced by mixing the ingredients.

Sucrose (Kishida Chemical Co., Ltd.)	20.0 wt %
Corn oil (Nacalai Tesque, Inc.)	5.0 wt %
Choline bitartrate (Tokyo Kasei Kogyo Co., Ltd.)	0.4 wt %
Corn starch (Nippon Starch Chemical Co., Ltd.)	39.1 wt %
AIN-76 vitamin (Oriental Yeast Co., Ltd.)	1.0 wt %
AIN-76 mineral (Oriental Yeast Co., Ltd.)	3.5 wt %
Cellulose (Oriental Yeast Co., Ltd.)	5.0 wt %
Casein (Wako Pure Chemical Industries, Ltd.)	25.0 wt %
Powder produced in Example 25	1.0 wt %

What is claimed is:

1. A liver function protecting or improving agent which comprises a plant of the family Saxifragaceae or an extract of the plant as an active ingredient.

2. The liver function protecting or improving agent according to claim 1, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraqa.

3. The liver function protecting or improving agent according to claim 2, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

4. The liver function protecting or improving agent according to claim 1, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

5. The liver function protecting or improving agent according to claim 4, wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

6. The liver function protecting or improving agent according to any of claims **1** to 5, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

7. The liver function protecting or improving agent according to any of claims 1 to 6, which is administered orally.

8. The liver function protecting or improving agent according to any of claims 1 to 7, wherein the liver function is a function affected by alcohol.

9. A food and drink which comprises a plant of the family Saxifragaceae or an extract of the plant.

10. The food and drink according to claim 9, which is useful for the protection or improvement of liver function.

11. The food and drink according to claim 10, wherein the liver function is a function affected by alcohol.

12. The food and drink according to any of claims 9 to 11, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

13. The food and drink according to claim 12, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

14. The food and drink according to any of claims 9 to 11, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

15. The food and drink according to claim 14, wherein the plant belonging to the genus Hydranqea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

16. The food and drink according to any of claims **9** to 15, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

17. A feed which comprises a plant of the family Saxifragaceae or an extract of the plant.

18. The feed according to claim 17, which is useful for the protection or improvement of liver function.

19. The feed according to claim 18, wherein the liver function is a function affected by alcohol.

20. The feed according to any of claims 17 to 19, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

21. The feed according to claim 20, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

22. The feed according to any of claims 17 to 19, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

23. The feed according to claim 22, wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

24. The feed according to any of claims 17 to 23, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

25. An additive for foods and drinks having liver function protecting or improving activity, which comprises a plant of the family Saxifragaceae or an extract of the plant.

26. The additive for foods and drinks according to claim 25, wherein the liver function is a function affected by alcohol.

27. The additive for foods and drinks according to claim 25 or **26**, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

28. The additive for foods and drinks according to claim 27, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

29. The additive for foods and drinks according to claim 25 or **26**, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

30. The additive for foods and drinks according to claim 29, wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

31. The additive for foods and drinks according to any of claims 25 to 30, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

32. A feed additive having liver function protecting or improving activity, which comprises a plant of the family Saxifragaceae or an extract of the plant.

33. The feed additive according to claim 32, wherein the liver function is a function affected by alcohol.

34. The feed additive according to claim 32 or **33**, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

35. The feed additive according to claim 34, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

36. The feed additive according to claim 32 or **33**, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

37. The feed additive according to claim 36, wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

38. The feed additive according to any of claims 32 to 37, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifraqaceae.

39. A method of screening for liver function protecting or improving agents, which comprises administering an alcohol and then a lipopolysaccharide to an animal to raise the blood GPT or GOT level of the animal, administering a test substance to the animal, and estimating the activity of the test substance to lower the blood GPT or GOT level of the animal.

40. The method according to claim 39, wherein the animal is a mammal.

41. The method according to claim 39 or **40**, wherein the lipopolysaccharide is derived from a microorganism belonging to the group of enteric bacteria.

42. The method according to any of claims 39 to 41, wherein the liver function is a function affected by alcohol.

43. A method of protecting or improving liver function in an animal, which comprises feeding the animal with the liver function protecting or improving agent according to any of claims 1 to 8 or the feed according to any of claims 17 to 24.

44. The method according to claim 43, wherein the animal is selected from the group consisting of livestock, poultry and cultivated fish.

45. A food and drink or feed for the protection or improvement of liver function which comprises a plant of the family Saxifragaceae or an extract of the plant as an active ingredient.

46. The food and drink or feed according to claim 45, wherein the plant of the family Saxifragaceae belongs to the genus Saxifraga.

47. The food and drink or feed according to claim 46, wherein the plant belonging to the genus Saxifraga is *Saxifraga stolonifera* Meerb.

48. The food and drink or feed according to claim 45, wherein the plant of the family Saxifragaceae belongs to the genus Hydrangea.

49. The food and drink or feed according to claim 48, wherein the plant belonging to the genus Hydrangea is *Hydrangea macrophylla* Seringe var. Thunbergii Makino or Hydrangeae Dulcis Folium.

50. The food and drink or feed according to any of claims 45 to 49, wherein the extract of the plant of the family Saxifragaceae is an alcoholic extract of the residue of an aqueous medium extract of the plant of the family Saxifragaceae.

51. The food and drink or feed according to any of claims 45 to 50, wherein the liver function is a function affected by alcohol.

* * * * *

US005081149A

United States Patent [19]

Ohmori et al.

[11] **Patent Number: 5,081,149**

[45] **Date of Patent: Jan. 14, 1992**

[54] **ANTIHEPATOPATHIC METHOD**

[75] Inventors: **Shinji Ohmori**, Okayama; **Kazumi Ogata**, Toyonaka; **Takahiro Sakaue**, Itami, all of Japan

[73] Assignee: **Senju Pharmaceutical Co., Ltd.**, Osaka, Japan

[21] Appl. No.: **588,097**

[22] Filed: **Sep. 21, 1990**

[30] **Foreign Application Priority Data**

Sep. 29, 1989 [JP] Japan 1-256370

[51] **Int. Cl.**[5] **A61K 31/24**

[52] **U.S. Cl.** **514/534**

[58] **Field of Search** 514/534

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

63-8337 1/1988 Japan .
2-255624 10/1990 Japan .
3-48626 3/1991 Japan .

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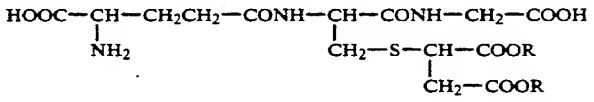
Primary Examiner—Stanley J. Friedman
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] **ABSTRACT**

The present invention relates to a useful antihepatopathic composition comprising a compound of the formula:



(wherein the R groups are the same or different and each means a hydrogen atom or a lower alkyl group) or a pharmaceutically acceptable salt thereof as an active ingredient.

1 Claim, No Drawings

ANTIHEPATOPATHIC METHOD

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an antihepatopathic agent. More particularly, it relates to a useful pharmaceutical composition for the prevention and treatment of hepatopathy which contains S-(α,β-dicarboxyethyl)glutathione which is found in the mammalian body, or an ester derivatives thereof, either in the free form or in a pharmaceutically acceptable salt form.

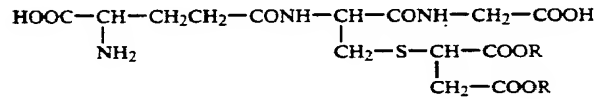
2. Description of the Prior Art

Many of the antihepatopathic agents so far used contain the group SH. Cysteine and glutathione are typical examples. However, while these agents produce a detoxicating effect owing to their active SH group, they have a disadvantage in that the active SH group reduces the efficacy of a drug or drugs used concomitantly.

The present inventors made intensive investigations in search of potent antihepatopathic compounds having no active SH group, hence having no such disadvantage as mentioned above and, as a result, found that S-(α,β-dicarboxyethyl)glutathione, which is a substance present in the mammalian body, and ester derivatives thereof unexpectedly have very potent antihepatopathic activity and at the same time have a high level of safety. The present invention has been accomplished on the basis of the above findings.

SUMMARY OF THE INVENTION

The invention provides an antihepatopathic composition, namely a pharmaceutical composition for the prevention and treatment of hepatopathy which comprises, as an active ingredient, a compound of the formula



(wherein the two R groups are the same or different and each is a hydrogen atom or a lower alkyl group) or a pharmacologically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

Among the active ingredients to be used in the composition according to the invention, S-(α,β-dicarboxyethyl)glutathione is a physiological substance discovered in the bovine crystalline lens by D. H. Calam and S. G. Waley [Biochem. J., 86, 226 (1963)]. At present, however, only a little is known about its pharmacological features. The present inventors have previously found that this compound has blood coagulation inhibiting and platelet aggregation inhibiting activities as well as antiinflammatory and/or antiallergic activity [Japanese Published Unexamined Patent Application (Kokai) No. 63-8337 and Japanese Patent Applications Nos. 1-79956 and 1-183484].

In the above formula, the two R groups are the same or different and each is a hydrogen atom or a lower alkyl group preferably containing 1 to 10 carbon atoms. The carbon chain in said alkyl group may be straight or branched or cyclic. Furthermore, said chain may contain a cyclic portion. Thus, as the alkyl group, there may be mentioned, among others, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, tert-butyl, sec-butyl, n-pentyl, 1-ethylpropyl, isopentyl and benzyl.

In the antihepatopathic composition according to the invention, the active ingredient compound may be in its free form or in the form of a pharmacologically acceptable nontoxic salt, for example an alkali metal salt, such as a sodium salt or potassium salt, or an alkaline earth metal salt, such as a calcium salt or magnesium salt. In cases where said compound is in a salt form, the carboxyl groups occurring in said compound may be either fully or partially involved in salt formation. Any of the possible salts can be used for preparing the composition according to the invention.

The antihepatopathic composition according to the invention may contain one of or a combination of two or more of the active ingredient compounds defined hereinabove, depending on the intended purpose and/or necessity.

The active ingredient compounds to be used in the antihepatopathic composition according to the invention can be produced by various means, for example in the following manner. Thus, S-(α,β-dicarboxyethyl)-glutathione can be extracted, isolated and purified from yeasts or the bovine crystalline lens, for instance, since it occurs there. Alternatively, S-(α,β-dicarboxyethyl)-glutathione can be synthesized by allowing an equimolar mixture of the starting materials glutathione and maleic acid in aqueous solution or in alcoholic aqueous solution to stand with warming or at room temperature for 1 to 2 days. The use of a maleic acid monoester or diester in lieu of maleic acid gives the corresponding S-(α,β-dicarboxyethyl)glutathione ester derivative. While the compounds synthesized in the above manner have a newly introduced asymmetric carbon atom in their molecules and hence involve optical isomers, both isomers as well as mixtures thereof can suitably be used in the practice of the invention.

The active compound to be used in the antihepatopathic composition of the present invention is a substance present in the body or an ester derivative thereof and therefore it is clear to be scarcely toxic, as evidenced by the data generated and shown in Test Example 2 which is described herein, hence is excellent in safety and can be advantageously used in various dosage forms against various hepatic disorders.

The antihepatopathic composition of the present invention effectively inhibits the onset of liver damage, whether acute or chronic, and prevents elevation of GOT, GPT and LDH values. It is, thus, of value in the prevention and treatment of acute or chronic hepatitis. It can also be used effectively for hepatocirrhosis. The antihepatopathic composition according to the invention controls the fall in liver TG as resulting from ingestion of alcohol and therefore is useful in the prevention and treatment of alcoholic liver disease as well. Furthermore, it can be used advantageously against drug-induced liver damage, for example the damage caused by acetaminophen.

In the prevention and/or treatment of various forms of hepatopathy, such as those mentioned above, the composition according to the invention is suitably applied either orally or parenterally depending on the disease to be treated. Usable dosage forms are, for instance, solid form preparations, such as tablets, granules, powders and capsules, and liquid form preparations such as injections. These preparations can be prepared by methods well known in the art. The preparations may contain, as necessary, those inert components,

auxiliaries and additives that are commonly used in the pharmaceutical practice, for example binders, disintegrants, thickeners, dispersants, reabsorption promoting agents, corrigents, flavors, buffers, surfactants, solubilizers, preservatives, emulsifiers, isotonizing agents, stabilizers and pH adjusting agents, each in an appropriate amount.

The dose of the active ingredient compound mentioned above may vary depending on the compound, patient's age and body weight, dosage form, symptom to be treated and other factors. Generally, however, it is recommendable that, in the case of an injection, for instance, about 10 to 500 mg be administered once daily to each adult human; in the case of peroral dosage forms, about 10 to 1,000 mg be administered several times daily to each adult.

The composition according to the invention may further contain another or other antihepatothic agents and/or another or other agents capable of producing other pharmacological effects unless the object of the invention becomes unattainable.

The following examples are further illustrative of the present invention.

TEST EXAMPLE 1

Test for pharmacological effects on hepatopathy

Method

After 24 hours of fasting, SD-strain male rats weighing 180 g were orally given 200, 400 or 800 mg/kg of S-(α, β-dicarboxyethyl)glutathione (hereinafter referred to as "DCE-GS" for short). A control group was given physiological saline. An hour later, 300 mg/kg acetaminophen was intraperitoneally administered to each rat for causing hepatopathy. Twenty-four hours after acetaminophen administration, blood samples were collected from the rats under pentobarbital anesthesia via the abdominal aorta and subjected to blood biochemistry tests. The results obtained are shown below in Table I.

TABLE I

Test substance	Dose (mg/kg)	Effects of DCE-GS on acetaminophen-induced hepatopathy in rats: s-GOT	s-GPT	LDH	s-TG
physiological saline	—	2545 ± 769	1289 ± 339	12467 ± 3002	27.3 ± 2.3
DCE-GS	200	1221 ± 179	499 ± 66	6029 ± 485	28.8 ± 5.0
	400	677 ± 219*	262 ± 89*	4873 ± 973*	36.1 ± 7.6
	800	438 ± 131*	133 ± 39*	3722 ± 595*	53.9 ± 10.4*

Each value indicate the mean ± standard error (n = 6 to 8).
Significance level relative to physiological saline: *$p < 0.05$.
Units are IU/liter for s-GOT and s-GPT; Wrobrewski units for LDH (lactate dehydrogenase); and mg/dl for s-TG (serum triglyceride).

Results

Administration of acetaminophen to rats induced hepatopathy; the s-GOT, s-GPT and s-LDH values increased and the s-TG value decreased.

DCE-GS dose-dependently suppressed the increases in s-GOT, s-GPT and s-LDH as well as the decrease in s-TG.

TEST EXAMPLE 2

Acute toxicity testing by intravenous administration

Acute toxicity testing was carried out by intravenous administration of DCE-GS to groups of 5 male ddy strain mice weighing about 20 g. The doses used were 100, 200, 400, 800 and 1,600 mg/kg (common ratio=2). The injection were adjusted to pH 7 with 1N sodium hydroxide. Neither deaths nor behavioral abnormalities were noted during 72 hours of observation.

SYNTHESIS EXAMPLE 1

S-(α, β-Dicarboxyethyl)glutathione

Glutathione (9.2 g) and 5.0 g of maleic acid are dissolved in 150 ml of water and the solution is allowed to stand at room temperature for 12 hours. The reaction mixture is sampled (one or two drops) and one drop of 0.01N I_2 test solution is added to the sample. After confirming, in this manner, that there is no more iodine consumption, 6.6 g of copper acetate (monohydrate) is added to the reaction mixture. If a precipitate, which is small in amount, is found, the precipitate is filtered off. The filtrate is concentrated to about 70 ml, ethanol is added to the concentrate, and the resulting blue copper salt precipitate is collected by filtration. This is recrystallized from water-ethanol. This copper salt is further dissolved in 200 ml of water, hydrogen sulfide is passed through the solution to cause precipitation of copper sulfide, and the precipitate is filtered off. The filtrate is concentrated under reduced pressure and ethanol is added to the residue, whereupon white crystals appear. The crystals are collected by filtration, washed with ethanol and recrystallized from water-ethanol to give about 9 g of the desired compound as white amorphous crystals (hygroscopic).

SYNTHESIS EXAMPLE 2

Sodium salt of S-(α, β-dicarboxyethyl)glutathione

S-(α, β-Dicarboxyethyl)glutathione (2 g) is dissolved in 40 ml of water, the solution is adjusted to pH 7 with 1N NaOH and then concentrated under reduced pressure at a temperature not exceeding 30° C. Ethanol is added to the concentrate and the resulting white crystals are collected by filtration and recrystallized from water-ethanol to give 2.1 g of the desired compound as a white crystalline powder.

SYNTHESIS EXAMPLE 3

Calcium salt of S-(α, β-dicarboxyethyl)glutathione

5-(α, β-Dicarboxyethyl)glutathione (2 g) is dissolved in 40 ml of water, 1 g of calcium carbonate is added to the solution, and the mixture is stirred with warming. When carbon dioxide gas evolution is no more observed, the excess of calcium is filtered off. The filtrate is concentrated under reduced pressure. Ethanol is added to the concentrate and the resulting white crystalline precipitate is collected by filtration and recrystallized from water-ethanol to give 2.2 g of the desired compound as a white crystalline powder.

SYNTHESIS EXAMPLE 4

Magnesium salt of S-(α, β-dicarboxyethyl)glutathione

S-(α, β-Dicarboxyethyl)glutathione (2 g) is treated with 1 g of basic magnesium carbonate in the same manner as mentioned above for the production of the calcium salt, to give 2.2 g of the magnesium salt.

SYNTHESIS EXAMPLE 5

Sodium salt of S-(diethyl-α, β-dicarboxyethyl)glutathione

Glutathione (9.2 g) and 5.6 g of diethyl maleate are dissolved in 150 ml of 30% (v/v) ethanol. The solution is adjusted to pH 6 with 2N sodium hydroxide and then stirred at 50° C. for about 5 hours. The reaction mixture is sampled (two drops) and one drop of 0.01N iodine test solution is added to the sample. When the color of iodine does not fade any more, gaseous hydrogen sulfide is passed through the reaction mixture. The mixture is allowed to stand overnight and then concentrated, whereby the hydrogen sulfide gas is distilled off. Water (150 ml) is added to the residue for dissolution of the residue. Copper acetate monohydrate (6.6 g) is added to and dissolved in the solution. The copper salt gradually precipitates out. The precipitate is collected by filtration, washed with water and suspended in 150 ml of water. Gaseous hydrogen sulfide is passed through the suspension with stirring for the formulation of copper sulfide. The copper sulfide is filtered off, the filtrate is concentrated, ethanol (200 ml) was added to the residue for dissolving the same, and the solution is adjusted to pH 6 by gradually adding an ethanolic sodium hydroxide solution, whereupon white crystals precipitate out. These are collected by filtration, washed with ethanol and dissolved in water for recrystallization. The solution is concentrated as far as possible and then ethanol is added. The resulting crystalline precipitate is collected by filtration and dried to give 8.5 g of S-diethyl-α, β-dicarboxyethyl)glutathione sodium salt. TLC on silica gel: Rf=0.28 (n-butanol-acetic acid-water=4:1:1).

SYNTHESIS EXAMPLE 6

Sodium salt of S-di-n-butyl-α, β-dicarboxyethyl)glutathione

Glutathione (9.2 g) and 7.5 g of di-n-butyl maleate are dissolved in 150 ml of 50% (v/v) ethanol and the reaction is carried out in the same manner as Synthesis Example 5. The solvent is then distilled off and the residue is dissolved in 150 ml of water. Addition of 200 ml of 3.3% aqueous copper acetate to the solution results in precipitation of the water-insoluble copper salt. The precipitate is collected by filtration, washed with water and suspended in 300 ml of 50% (v/v) ethanol. Hydrogen sulfide is passed through the suspension with stirring for the formation of copper sulfide. The copper sulfide is filtered off, the filtrate is concentrated for the removal of hydrogen sulfide. The concentration is dissolved again in 150 ml of 50% (v/v) ethanol, and the solution is adjusted to a pH of about 6 by addition of 2N sodium hydroxide solution and then concentrated. Ethanol, acetone and isopropyl ether are added to the concentrate. The resulting white crystalline precipitate is collected by filtration, washed with acetone and dried to give 9.7 g of S-(di-n-butyl-α,β-dicarboxyethyl)-glutathione sodium salt as hygroscopic crystals. TLC on silica gel: Rf=0.40 (n-butanol-acetic acid-water=4:1:1).

SYNTHESIS EXAMPLE 7

Calcium salt of S-(di-n-butyl-α,β-dicarboxyethyl)glutathione

The procedure of Synthesis Example 6 was followed using calcium carbonate in lieu of the 2N sodium hydroxide. Addition of acetone to the concentration residue gives white crystals. They are recrystallized from ethanol-acetone to give 7.5 g of S-(di-n-butyl-α, β-dicarboxyethyl)glutathione calcium salt.

SYNTHESIS EXAMPLE 8

Sodium salt of S-(monoethyl-α,β-dicarboxyethyl)glutathione

Glutathione (9.2 g) and 4.5 g of monoethyl maleate are dissolved in 150 ml of water, the solution is adjusted to pH 6.0 with 2N sodium hydroxide, and the reaction is carried out in the same manner as Synthesis Example 5. The reaction mixture is concentrated, ethanol is added to the residue, and the resulting precipitate white crystals are collected by filtration and dissolved in water for recrystallization. The aqueous solution is concentrated and ethanol is added to cause crystallization. Yield 8.0 g. TLC on silica gel: Rf=0.17 (n-butanol-acetic acid-water=4:1:1).

DOSAGE FORM EXAMPLE 1

Peroral tablets	
DCE-GS calcium salt	100 mg
Lactose	80 mg
Starch	17 mg
Magnesium stearate	3 mg

Tablets are produced by a conventional method using the above materials, the quantities given above being for one tablet. The tablets may be sugar-coated as necessary.

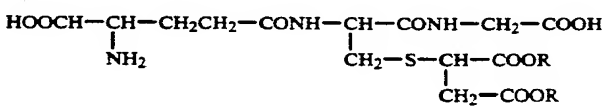
DOSAGE FORM EXAMPLE 2

Injectable solution	
DCE-GS sodium salt	2.5 mg
Sodium chloride	0.6 mg
Distilled water for injection	100 ml

The above ingredients are mixed up and sterilized by bacterial filtration. The filtrate is distributed in 2-ml portions into glass ampoules and the ampoules are sealed.

What is claimed is:

1. A method for the treatment of hepatopathy which comprises administering to a human in need of such treatment an anti-hepatopathy effective amount of a compound of the formula:



wherein the R groups are the same or different and each means a hydrogen atom or a lower alkyl group or a pharmaceutically acceptable salt thereof as an active ingredient.

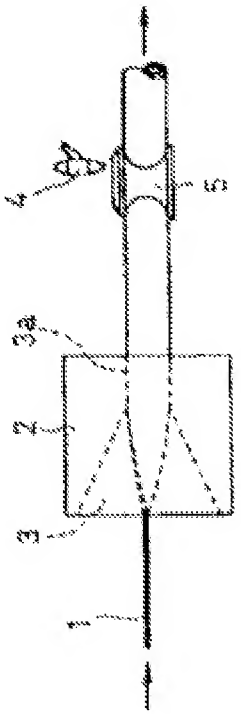
* * * * *

MANUFACTURE OF METALLIC WELDED TUBE

Publication number: JP3207523
Publication date: 1991-09-10
Inventor: NAKAKO TAKEFUMI; SHIMADA YOSHIAKI; MIURA NORIMASA; TAKEZOE AKINOBU
Applicant: NISSHIN STEEL CO LTD
Classification:
- international: ***B21C37/08; B21B37/20; B23K26/00; B23K26/20;*** **B21C37/08; B21B37/16; B23K26/00;** (IPC1-7): B21C37/08; B23K26/00
- European:
Application number: JP19900049549 19900302
Priority number(s): JP19900049549 19900302; JP19890278507 19891027

Abstract of **JP3207523**

PURPOSE:To form the diameter of a through hole on the outlet side at the same time in the form of a tube of the same outer diameter by passing a metallic plate through a die provided with a through hole of a circular section section having a tapered part in which a diameter is reduced from the inlet side to the outlet side thereof. CONSTITUTION:A metallic plate 1 is passed through a through hole 3 of a circular section having a tapered part in which the diameter is reduced from the inlet side of the die to the outlet side thereof. In the process in which this through hole 3 is passed, the metallic plate 1 is bent along the tapered part and elongated in the lengthwise direction, therefore, when the flat metallic plate 1 is passed through the through hole 3 of the die 2, it can be formed at the same time into the form of a pipe having the same outer diameter with the diameter of the through hole on the outlet side. Thereafter, as the tube form is held by squeeze rollers, the butted part of both side edges of the strip is TIG-welded. In this way, when the outer diameter of the metallic pipe to be manufactured is changed, die changing can be performed in a short time and easily.



CLAIMS

[Claim(s)]
[Claim 1]A presentation of a source of total nitrogen (protein and free amino acid) is 36 to 44 % of the weight as a peptic hydrolysate of animal protein, A constituent effective in a hepatopathy patient who is 18 to 22 % of the weight as a peptic hydrolysate of vegetable protein, and is characterized by a free amino acid containing at a rate of the following presentation.
A composition range of a free amino acid (weight percent per nitrogen source)
glutamic acid 15.7 -19.25 leucine . 5.45- 6.70 Valine 4.35-5.35. Isoleucine 3.65- 4.50 The sum of phenylalanine and tyrosine 1.45- 1.80 Lysine 1.25- 1.60 Threonine 1.25- 1.60 The sum of methionine and cystine 1.10-1.35. Histidine 1.10- 1.35 Tryptophan 0.55-0.70[Claim 2]The constituent according to claim 1, wherein vegetable protein is wheat gluten.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the enzymatic hydrolysate prepared from animals-and-plants protein, and the constituent which blends amino acid.
More particularly, it is related with the constituent which contains the hydrolyzate and the free amino acid of animal protein and wheat gluten at a specific rate.

[0002]Since a liver function can supply a required nutrient, without applying a burden to liver to the patient who fell, the constituent concerning this invention, It is used by a medical type, and also it can also be used by eating-and-drinking article types, such as a drink, foodstuffs, protective foods, a food for specified health use, and drinkable preparations, and not only a liver failure patient but a liver function can use it widely to a healthy person again also as opposed to the patient who fell for the prevention thru/or health.
[0003]
[Description of the Prior Art]As for the dietary therapy to the patient to whom the liver function fell, the meal of high energy and a high-protein presentation serves as the foundation. As disorders of amino acid metabolism and the protein metabolic turnover in liver failure become clear these days, The necessity for a nutrition therapy of having taken the amino acid composition of foodstuffs into consideration has been discussed in order to aim at correction of the imbalance of the plasma free amino acid seen by liver failure (JJPEN 3:109, 1981 besides Watanabe Meiji). Protein must fully be supplied, without causing the hepatic coma also to the example of protein intolerance in this case.
[0004]About the manifestation mechanism of the hepatic encephalopathy by which liver failure is characterized. the abnormalities of the plasma free amino acid pattern in a serious illness hepatopathy, especially branched chain amino acid (valine.) A rise of reduction and aromatic amino acid (phenylalanine, tyrosine) of leucine and isoleucine, tryptophan, and methionine is characteristic, and it is clear that the abnormalities of an intracranial amine metabolic turnover originating in disorder of this plasma free amino acid pattern are the main causes of hepatic encephalopathy.
[0005]Reduction of the branched chain amino acid in blood at the time of liver failure (valine, leucine, isoleucine) is for the insulin which increased by a hepatopathy to make incorporation of the branched chain amino acid in the muscular tissue or fat tissue increase.
The increase in the aromatic amino acid in blood (phenylalanine, tyrosine), and tryptophan A liver and protein catabolism sthenia of a muscle, It is thought [the fall of the protein composition ability of a liver, and] that it is further based on the fall of the aromatic amino

acid (phenylalanine, tyrosine) in liver and the throughput of tryptophan.
Therefore, it is constituted from the drugs for liver failure by the free amino acid which enlarged the ratio (Fischer ratio) of branched chain amino acid (valine, leucine, isoleucine) and aromatic amino acid (phenylalanine, tyrosine) remarkable.
[0006]About the digestion of the protein which is one of the 3 Oe nutrient, and amino acid, it is known within the intestinal tract that the ingestion of the free amino acid in that proteinic low molecule peptide is promptly absorbed from an amino acid simple substance and dietary therapy will give stress remarkably to a patient.
[0007]
[Problem(s) to be Solved by the Invention]This invention supplies the source of protein which not only the patient of liver failure but a liver function needs also to the patient who fell in view of such the actual condition, without applying a burden to liver, And it is made in order to newly develop the outstanding constituent which can be used in dietary therapy over a long period of time without giving stress to a patient.
[0008]
[Means for Solving the Problem]To achieve the above objects, a point which normalizes a metabolic turnover and an internal secretion moving state by keeping plasma free amino acid balance normal as a result of inquiring from every direction is noted for the first time, From the necessity for a nutrition therapy of having taken amino acid composition of foodstuffs into consideration in order to aim at correction of plasma amino acid imbalance at the time of a decreased liver function. Hydrolyzate of animality and vegetable protein was used as a source of protein, and it discovered that a constituent suitable not only for a patient of liver failure but a patient to whom a liver function fell for dietary therapy was obtained for the first time by moreover mixing a free amino acid.
[0009]And as a result of inquiring further from every direction, acquire knowledge that hydrolyzate of animality and vegetable protein and a constituent with a free amino acid are very effective as a source of protein, and it is based on these useful new knowledge, As a result of repeating research, it results in completion of this invention at last, and this invention becomes considering a specific constituent containing proteinic hydrolyzate and a free amino acid as fundamental technical thought. Hereafter, this invention is explained in full detail.
[0010]In a constituent concerning this invention, a point which uses hydrolyzate and a free amino acid of animality and vegetable protein as a source of protein is one of the important features. In this invention, protein of vegetable origin which protein of animal origin acquired considering flesh of animals, a chicken, fish meat, cow's milk, an egg, etc. as a raw material is widely used as animal protein, and is obtained considering wheat, a soybean, rice, etc. as a raw material as vegetable protein is used widely.
[0011]As a result of repeating research about these ingredients, and inside of these ingredients, When each peptic hydrolysate of wheat gluten is used as refining meat protein and vegetable protein especially as animal protein, An effective thing was traced when such hydrolyzates blended at a rate of 36-44, and 18-22 with weight percent of a source of total nitrogen in a constituent (protein and free amino acid). And in order to improve validity as a constituent for patients in which a liver function fell further, blending a free amino acid checked a good thing. In that case. a free amino acid -- weight percent of a source of total nitrogen -- respectively -- glutamic acid . 15.7 - 19.25 leucine 5.45-. 6.70 Valine 4.35-5.35. Isoleucine 3.65- 4.50 The sum of phenylalanine and tyrosine 1.45- 1.80 Lysine 1.25- 1.60 Threonine 1.25- 1.60 The sum of methionine and cystine 1.10-1.35. Histidine 1.10- 1.35 Tryptophan An effective thing was traced when contained at a rate of 0.55-0.70. And it succeeded also in determination of the amount of optimum blending of ** (** et al.). As a result, a rate of a free amino acid over all the sources of protein (a source of total nitrogen: protein and amino acid) became about 40%, it became clear that stress was not given to a patient at the time of an ingestion in dietary therapy, and it resulted in completion of this invention article at last.
[0012]An ingredient of hydrolyzate of protein used in this invention points out all ingredients of a peptic hydrolysate of animal protein of the various kinds and protein of vegetable origin of wheat gluten and others which were manufactured in accordance with a conventional method.

[0013]A constituent concerning this invention makes an active principle hydrolyzate and a free amino acid of various kinds of animality and vegetable protein which were described above as a source of protein, moreover, comes to carry out specific amount combination as mentioned above, and is used as an eating-and-drinking article or medicine. An active principle of ** (** et al.) can be used as it is, or it uses together with other foodstuffs thru/or a food composition, and can be suitably used in accordance with a conventional method. As description of this constituent, any of a solid state (powder, granularity, others), paste state, and liquefied thru/or suspended state may be sufficient.
[0014]When using this constituent as medicine, in accordance with a conventional method, it can be considered as a tablet, a granule, powders, a capsule, powder medicine, liquids and solutions, drinkable preparations, etc., and there is no internal use and enteral administration can be carried out.
[0015]or [that there is no toxicity since an active principle concerning this invention is the natural product origin] -- or it is very low and very safe (LD_{50}>60 g/kg taking orally: rat). What is necessary is to refer to enteral feeding of the conventional known and just to define it according to a patient's condition, when using as medicine, and there is no exceptional restriction in particular and the dose should just also make it be the same as that of the usual eating-and-drinking article, when using as an object for prevention, an object for health, or an eating-and-drinking article.
[0016]
[Function]The protein needed while a liver function secures energy to the patient who fell can be taken in by using the constituent containing the hydrolyzate and the free amino acid of animal protein and wheat gluten as protein which is one of the 3 Oe nutrient. The plasma GOT and GPT, an insulin-like growth factor (IGF), and an amino gram are normalized as the result, and a liver function is maintained or improved.
[0017]Hereafter, in order to clarify this invention further, the example of manufacture of the constituent of this invention is given as an example, and subsequently the example of an examination is shown.
[0018]
[Example]After being suspended in water and adding refining pork protein at a rate of the protein 500g per cow pepsin 1.6g, chloride was added and pH was adjusted to 2.1. Then, protein was hydrolyzed by stirring at 37 ** for 36 hours. On the other hand, after suspending wheat gluten in water and adding the protein 500g per cow pepsin 1.0g, chloride was added and pH was adjusted to 2.1. Then, protein was hydrolyzed by stirring at 37 ** for 24 hours.
[0019]About each hydrolyzate solution, it cooled, after heating for 10 minutes at 100 **, respectively. After adding ethanol so that the concentration of the ethanol of the solution may be 80%, the ** exception carried out, the filtrate was condensed with the freeze drying method, and each hydrolyzate concerning this invention was manufactured.
[0020]The constituent effective in a hepatopathy patient was manufactured according to the combination shown in the following table 1.
[0021]
[Table 1]

精製豚肉たんぱく質のペプシン加水分解物	８．６４％
小麦グルテンのペプシン加水分解物	４．３２％
グルタミン酸	３．７８％
ロイシン	１．３１％
バリン	１．０５％
イソロイシン	０．８７％
フェニルアラニン	０．３５％
リジン	０．３１％
スレオニン	０．３１％
メチオニン	０．２６％
ヒスチジン	０．２６％
トリプトファン	０．１３％
コーン油	２８．８　％
デキストリン	４３．１　％
ビタミン類	３．４８％
ミネラル類	３．０２％

[0022]
[Test Example(s)]In order to check the validity of a constituent effective in a hepatopathy patient, the model rat of a hepatopathy was created first and the effect was investigated by the animal experiment.
[0023]Each protein hydrolyzate and the free amino acid which were manufactured by the above-mentioned method were mixed at a rate of the following table 2 according to the ingredient and composition ratio of this invention, and the constituent for rats was manufactured. The constituent mixed at a rate of the following table 3 as comparison feed for rats (contrast casein composition thing) was manufactured.
[0024]
[Table 2]

肝障害に有効な組成物　（ラット飼料）

成　　分		（g／全組成物100ｇ）
精製豚肉たんぱく質のペプシン加水分解物		４．８　ｇ
小麦グルテンのペプシン加水分解物		２．４　ｇ
遊離アミノ酸		
	グルタミン酸	２．１　ｇ
	ヒスチジン	０．１５ｇ
	イソロイシン	０．４９ｇ
	ロイシン	０．７３ｇ
	バリン	０．５８ｇ
	トリプトファン	０．０８ｇ
	リジン	０．１７ｇ
	スレオニン	０．１７ｇ
	フェニルアラニン	０．２０ｇ
	メチオニン	０．１５ｇ
	βーコーンスターチ	７４．６　ｇ
共	大豆油	４．３９ｇ
通	セルロース粉	４．３９ｇ
成	ミネラル混合物（注１）	３．５２ｇ
分	ビタミン混合物（注１）	０．８８ｇ
（注2）	塩化コリン	０．１３ｇ
		１００ｇ

（注１）Rogers, Q. R. and Haper, A. E.
Amino Acids Diets and Maximal Growth in Rat,
Journal of Nutrition, 87, 267(1965)

（注２）National Research Council
Nutrient Requirements of Laboratory Animals, pp23-75,
National Academy of Sciences, Washington, D. C. (1975)

[0025]
[Table 3]

対照カゼイン組成物　（ラット飼料）

成　　分		（g／全組成物100ｇ）
カゼイン		７．２　ｇ
遊離アミノ酸		
	グルタミン酸	２．１　ｇ
	ヒスチジン	０．１７ｇ
	イソロイシン	０．２８ｇ
	ロイシン	０．４１ｇ
	バリン	０．３３ｇ
	トリプトファン	０．０８ｇ
	リジン	０．３９ｇ
	スレオニン	０．２８ｇ
	フェニルアラニン	０．４４ｇ
	メチオニン	０．３３ｇ
	β－コーンスターチ	７４．６　ｇ
共	大豆油	４．３９ｇ
通	セルロース粉	４．３９ｇ
成	ミネラル混合物（注１）	３．５２ｇ
分	ビタミン混合物（注１）	０．８８ｇ
（注２）	塩化コリン	０．１３ｇ
		１００ｇ

（注１）Rogers, Q. R. and Haper, A. E.
Amino Acids Diets and Maximal Growth in Rats,
Journal of Nutrition, 87,267(1965)

（注２）National Research Council
Nutrient Requirements of Laboratory Animals, pp23-75,
National Academy of Sciences, Washington, D. C.(1975)

[0026]The model rat of a hepatopathy was created as a galactosamine hepatopathy animal in accordance with the conventional method.

[0027]Hereafter, each animal experiment compared about the effect as opposed to [which were prepared according to the ingredient and composition ratio of this invention / the constituent (Table 2) and comparison feed (Table 3)] a normal rat and a galactosamine hepatopathy rat.

[0028]The plasma GOT and GPT after administration was measured for the test meal for seven days in order to investigate the effect over a liver function. The result was shown in the following table 4. Especially, compared with the casein foods administration group of contrast, the fall was accepted in the constituent administration group of this invention, and the plasma GOT and GPT showed the liver function improvement effect. Each plasma amino gram of the essential amino acid at that time and nonessential amino acid was shown in drawing 1 and drawing 2.

[0029]

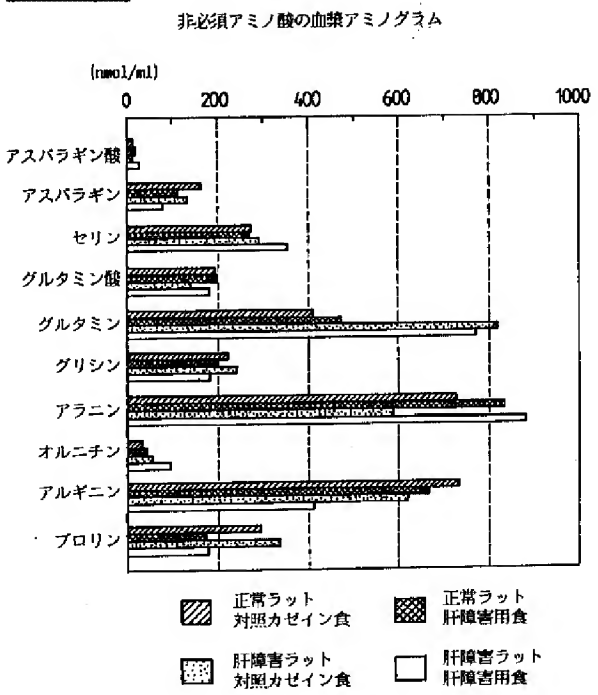
[Table 4]

	GOT (IU／l) SEM	GPT (IU／l) SEM
正常ラット 対照カゼイン食	60.1±1.82	14.2±1.20
正常ラット 肝障害用食	51.8±0.95	18.6±2.51
肝障害ラット 対照カゼイン食	72.8±2.85	36.5±1.21
肝障害ラット 肝障害用食	57.3±1.92	15.1±1.82

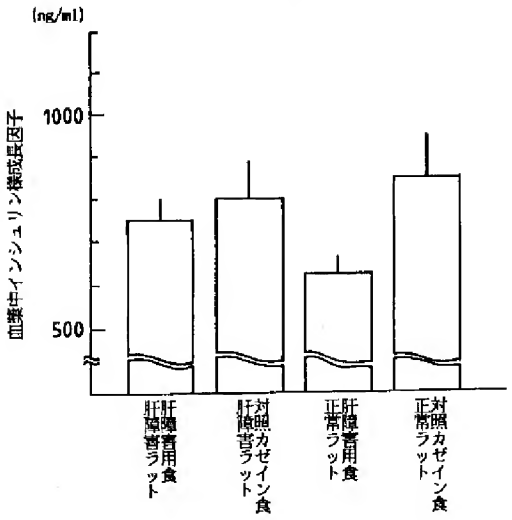
[0030]The amino acid balance in blood collapses at the time of liver failure, and it is known that especially branched chain amino acid will decrease in number. As a result of this administration experiment, in the constituent administration group of this invention, descent of phenylalanine and tyrosine which are the rise and aromatic amino acid of isoleucine, leucine, and valine which are the branched chain amino acid in blood was accepted, and plasma amino acid balance was normalized.
[0031]Since it was known that an insulin will increase in connection with a hepatopathy, it measured about change of the plasma insulin-like growth factor (IGF). The result was shown in drawing 3.
[0032]In the constituent administration group of this invention, the falls of the insulin-like growth factor content were accepted to be also a hepatopathy rat and a normal rat, and the liver function improvement effect was shown.
[0033]So that more clearly than the result of the animal experiment of the model rat of the above hepatopathy, By using the constituent containing the hydrolyzate and the free amino acid of the animal protein of this invention, and wheat gluten, the eating-and-drinking article which a hepatopathy patient treats and maintains, or the product with validity very high as drugs was completed.
[0034]
[Effect of the Invention]It became possible for side effects not to have a diet therapy to the therapy of liver disease made difficult over a long period of time, either, and to treat and maintain safely and effectively by this invention.

DRAWINGS

[Drawing 2]

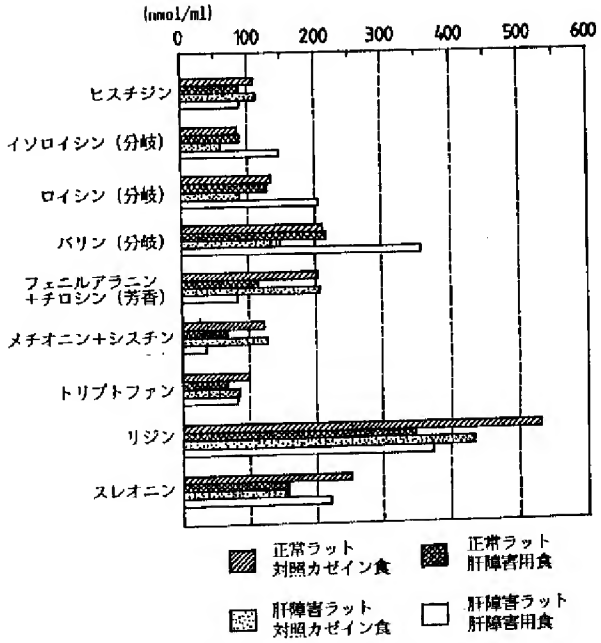


[Drawing 3]



[Drawing 1]

必須アミノ酸の血漿アミノグラム

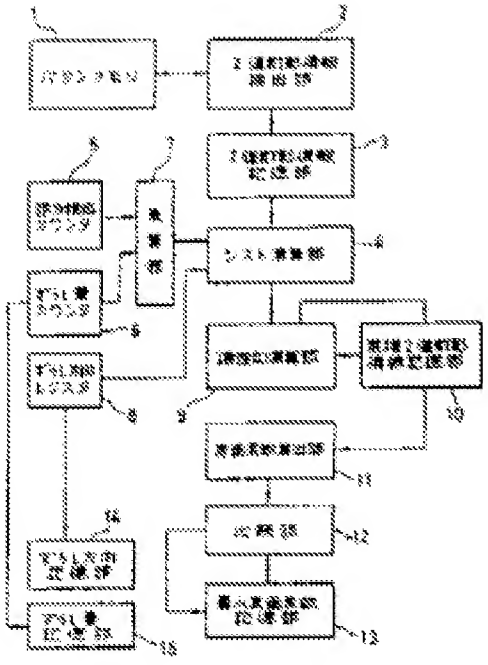


METHOD AND DEVICE FOR DETECTING TILT OF DOCUMENT PICTURE

Publication number:	JP3090980
Publication date:	1991-04-16
Inventor:	TSUJI YOSHITAKE
Applicant:	NIPPON ELECTRIC CO
Classification:	
- international:	***G06K9/32;*** **G06K9/32;** (IPC1-7): G06K9/32
- European:	
Application number:	JP19890226944 19890831
Priority number(s):	JP19890226944 19890831

Abstract of **JP3090980**

PURPOSE:To detect the tilt of a document picture in a stable and easy way by detecting the direction where the projection, i.e, the width of the black picture element showing a character row and a table frame when the character row and a table, etc., are projected in the tilt direction. CONSTITUTION:A pattern memory 1 stores the document pictures as the quantized picture information. A binary projection information extracting part 2 sets (m) pieces of partial information (i) of a fixed size in the prescribed direction, scans each partial area (i), and extracts the binary projection information showing the presence/absence of black picture elements on each scan line to store them in sequence into a binary projection information storage part 3. Then the adjacent binary projection information are shifted in steps in the vertical and horizontal shift direction by a prescribed extent and read out. At the same time, an OR is obtained among plural binary projections and the cumulative binary projection information are extracted. Then the shift value is changed for detection of the shift value and the shift direction where the number of black picture elements of the cumulative binary projection information is minimized. Thus the tilt of a document picture can be detected in an easy and stable way.



CLAIMS

[Claim(s)]
[Claim 1]A hepatocellular proliferation accelerator containing 11-deoxo glycyrrhizic acid or its water soluble salt as an active principle.
[Claim 2]A hepatopathy inhibitor containing 11-deoxo glycyrrhizic acid or its water soluble salt as an active principle.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the drugs which promote growth of hepatocytes, and the drugs which prevent a hepatopathy.
[0002]
[Description of the Prior Art]Conventionally, an insulin, PDGF, EGF, collagen, etc. are known as a hepatocellular proliferation accelerator, and vitamin E is known as a liver protecting agent. On the other hand, various pharmacological actions, such as antiulcer, anti-inflammation, and antiallergic, are shown for many years, and also as for the glycyrrhizin which is an active principle of the glycyrrhiza known as a crude drug, as for the operation which makes a hepatopathy recover, a certain thing is known. Some of GURICHIRURECHIN and other derivatives which are obtained from glycyrrhizin may show the same pharmacological action as glycyrrhizin, and they may show a peculiar pharmacological action depending on a compound. However, it is not reported that 11-deoxo glycyrrhizic acid is useful to hepatocellular proliferation promotion or prevention of a hepatopathy.
[0003]
[Problem(s) to be Solved by the Invention]The purpose of this invention is to provide a new hepatocellular proliferation accelerator and a hepatopathy inhibitor out of a glycyrrhizin derivative.
[0004]
[Means for Solving the Problem]This invention provides a new hepatocellular proliferation accelerator and a hepatopathy inhibitor which contain 11-deoxo glycyrrhizic acid or its water soluble salt as an active principle.
[0005]
[Function]11-deoxo glycyrrhizic acid or its water soluble salt (these may be hereafter called 11-deoxo glycyrrhizin in the meaning containing ********), Although it is little, it is the quality of a natural product contained in glycyrrhiza, and when extracting glycyrrhizin from glycyrrhiza, it is extracted with glycyrrhizin. Although the use as drugs with few side effects was expected, the pharmacological action is seldom studied. Of course, about the operation on hepatocytes or a liver function, it was strange. In the healthy animal which matured, although hepatocytes are in the hardly increased state of rest, when an obstacle arises in an organization and a necrosis of a cell and omission take place to it, they tend to start growth quickly, and it is going to recover them with the actual condition. Generally this hepatocellular proliferation becomes the most active in 30 to 40 hours, decreases after that and goes. Functional recovery of the hepatocytes by it can usually be checked in the protein synthesis ability of hepatocytes, and the protein content of hepatocytes. If 11-deoxo glycyrrhizin is prescribed for the patient after making a rat produce a hepatopathy experimentally, improvement in protein synthesis ability and the increase in the protein content in hepatocyte more remarkable than the case where 11-deoxo glycyrrhizin is not prescribed for the patient will be accepted. Therefore, it turns out that 11-deoxo glycyrrhizin has the operation which promotes recovery of hepatocytes notably.
[0006]If the hepatocyte which constitutes hepatocytes is seen with a chromosomal level, a tetraploid cell occupies two thirds of all the hepatocytes, and the diploid cell occupies one third, but if carbon tetrachloride processing is performed, omission of a tetraploid cell will occur selectively. These omission are usually produced about 7 hours after carbon tetrachloride administration. However, if a carbon tetrachloride is administered hypodermically to a rat and 11-deoxo glycyrrhizin is prescribed for the patient in 2 hours, omission of a tetraploid cell will be prevented thoroughly. This shows that 11-deoxo glycyrrhizin has the outstanding hepatopathy prevention operation. 11-deoxo glycyrrhizin is quality of a natural product contained in the glycyrrhiza extract as mentioned above, and moreover, since a glycyrrhiza extract is used as a seasoning or a drugs raw material for many years, even if there is toxicity of 11-deoxo glycyrrhizin, it is imagined as a very low thing.
[0007]11-deoxo glycyrrhizin used for the hepatocellular proliferation accelerator and hepatopathy inhibitor of

this invention can be easily manufactured from glycyrrhizin with the manufacturing method indicated to JP,60-38392,A. That is, chloride is made to react to zinc amalgam in the glycyrrhizin solution obtained by dissolving glycyrrhizin in dioxane, a tetrahydro franc, acetone, etc., and if the glycyrrhizin in a solution is returned from the hydrogen to generate, 11-deoxo glycyrrhizic acid will be obtained. If alkali metal hydroxide, an ammonia solution, etc. neutralize this, 11-deoxo glycyrrhizin acid chloride can be obtained. As a desirable example of usable 11-deoxo glycyrrhizin acid water solubility salt, among the drugs of this invention are alkali metal salt, such as sodium salt and potassium salt, ammonium salt, etc.

[0008]11-deoxo glycyrrhizin as a hepatocellular proliferation accelerator and a hepatopathy inhibitor administers orally, and also can also be used in the form of parenteral administration, such as a suppository or injections. Pharmaceutical preparation-ization is 11-deoxo glycyrrhizin independent and with or a vitamin, hormone, and a substance effective in another enzyme and hepatopathies therapy. It can be made powder medicine, a granule, a tablet, a capsule, syrups, etc. using arbitrary excipients, a binding material, disintegrator, lubricant, a diluent, stabilizer, etc. if needed. In the case of about 5-500mg/[a day and] and injection, a suitable dose is about 0.5-50mg/day by the case where it usually administers orally although it changes with condition, age, etc. It is.

[0009]

[Example]

Example 1: administering hypodermically 2.5ml/kg of a 20 w/v% carbon tetrachloride and olive-oil solutions, and behind 6 weeks-old rat (Wister system; male) which 11-deoxo glycyrrhizin to the hepatocyte protein synthesis inhibition at the time of an acute carbon tetrachloride hepatopathy made abstain from food for effect 24 hours, Glycyrrhizic acid or 11-deoxo glycyrrhizic acid was injected intraperitoneally 200 mg/kg 2 hours afterward. The control group was medicated only with the physiological saline. Hepatocytes were separated by the collagenase flowing-back method in 2 more hours, and the rate of cell survival was measured. Then, they are a $1x10^4$ individual / dish to the dish for tissue cultures (35 mm in diameter) about isolation hepatocytes. It sprinkled at a rate (1 sample 60 dish), it added each 1 ml of WE culture media, and culture was started within the 5% carbon dioxide incubator. Culture-medium exchange was performed 4 hours after the culture start. It was considered as 1 group 3 dish, ^{3}H-leucine (2 microcurie [ml] /, Amersham, 70 Ci/mmol) was added at each [of 20 hours, 40 hours, and 60 hours after] time, and protein synthesis ability was investigated. In this case, the culture medium was changed into Leu - Dalecco's modifiedEagle medium (10%FBS, 0.001%Insulin) 2 hours before prescribing ^{3}H-leucine for the patient. The part was isolated preparatively to protein determination and site flowmetry after the incubation of 2 hours, respectively. it dissolved in 0.4%SDS 100microl, and retained material drawing was settled in the 10%TCA-0.1M pyrophosphate -- the after filter trap was carried out and the radiation count was measured. Protein determination was performed in accordance with the Bio-Rad Protein Assey method.

[0010]Incorporation of ^{3}H-leucine 68 hours after a culture start and the measurement result of a protein volume are shown in Table 1. By carrying out carbon tetrachloride processing of the rat, the obstacle arose in hepatocytes and 30% of hepatocytes had fallen out. By a glycyrrhizic acid administration group, the improvement in protein synthesis ability was not accepted so that clearly from the uptake quantity of ^{3}H-leucine of Table 1, but by 11-deoxo glycyrrhizic acid administration group, it reached by 5.45 times the control group, and advanced recovery of a hepatic function was shown. In the case of glycyrrhizic acid, in the case of 11-deoxo glycyrrhizic acid, the maximum of protein synthesis is 70 hours after a culture start 48 hours after a culture start, and both protein synthesis decreased after that.

[0011]

Uptake quantity of table 1 ^{3}H-Leu Protein volume (cpm/cell) (mug/cell) control group untaken a measure 450 Control group 320 166 glycyrrhizic-acid administration group 466 187 11-deoxo glycyrrhizic acid administration group 17646 227[0012]The result of protein synthesis promotion has appeared also in the intracellular protein volume, and came to contain 1.4 times as many proteins compared with the control group by 11-deoxo glycyrrhizic acid administration group. When the examination same about 11-deoxo monoammonium salt glycyrrhizate and dipotassium salt as the above was done, the same result as the case where 11-deoxo glycyrrhizic acid is used was obtained.

[0013]Example 2 : After performing carbon tetrachloride processing to a rat like the case of the measurement example 1 of serum transaminase (GOT and GPT), 0.3 ml collected blood at a time 0, 10, and 24 or 48 hours afterward, it centrifuged for 10 minutes at 3000 rpm, the blood serum was extracted, and the transaminase (GOT, GPT) in a blood serum was measured with the conventional method. The control group prescribed only the physiological saline for the patient. GOT of 24 hours after and GPT of 10 hours after are shown in Table 2. Although the transaminase in a blood serum was generally used also for the clinical laboratory test as an index of a hepatopathy, it was checked that this is notably controlled by administration of 11-deoxo glycyrrhizic acid.

[0014]

Table 2GOT(KU/ml) GPT (international unit/ml) control group untaken a measure 84.6 44.8 control groups 2482**63.2 3273**40.0 glycyrrhizic-acid administration group Dose :. [100 mg/kg 3040.8 (7.1)]dose: -- 200

mg/kg 2549.8**60.1 (-). 2091.6**70.6(36.1)11-deoxo glycyrrhizic acid administration group dose: -- 100 mg/kg 1323.0 (59.6) dose: -- 200 mg/kg 1977.0**56.5 (79.6) 277.6**43.0 (91.5). Notes: The numerical value in a parenthesis is a control rate (%) of a control group standard.

[0015]Performing carbon tetrachloride processing to the rat like the case of example 3 Example 1, 11-deoxo glycyrrhizic acid was used as the physiological saline solution 1%, and injected kg intraperitoneally in 400mg /2 hours after carbon tetrachloride administration. The control group prescribed only the physiological saline for the patient. 22 hours afterward, liver was extracted and the nucleus was isolated by the hypertonicity sucrose method. Fluorescent staining of the isolation core was carried out by iodo-ized propidium, and karyotype analysis was conducted by site flowmetry. A result is as being shown in Table 3, and omission of tetraploid hepatocytes were thoroughly prevented by administration of 11-deoxo glycyrrhizic acid.

[0016]

table [as] somatic cell (32 times many %) tetraploid cell (%) control group untaken a measure 38.1 60.8 control group 66.4 32.5 11-deoxo glycyrrhizic acid administration group 24.0 74.9 -- in addition,When the examination same about 11-deoxo monoammonium salt glycyrrhizate and dipotassium salt as the above was done, the same result as the case where 11-deoxo glycyrrhizic acid is used was obtained.

[0017]

[Effect of the Invention]As mentioned above, according to this invention, a new hepatocellular proliferation accelerator and a hepatopathy inhibitor are provided, and it can contribute to a hepatopathy patient's recovery.